UNIVERSITY OF ALBERTA

STUDY ON THE TISSUE HARDENING PROBLEM OF REFRIGERATED RAW PRE-PEELED POTATOES

by

TIPAWAN THONGSOOK ©

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled STUDY ON TISSUE HARDENING PROBLEM OF REFRIGERATED RAW PRE-PEELED POTATOES submitted by Tipawan Thongsook in partial fulfillment of the requirements for the degree of Master of Science in Food Science and Technology.

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ABSTRACT

The occurrence of hard tissues in pre-peeled refrigerated potatoes results in hard lumps on cooking, which do not break down on mashing. Studies were conducted on pre-peeled, sliced potatoes to determine the possible causes of the hard tissues and the mechanisms by which they are formed. The factor studies were potato moisture content, antibrowning agents, and packaging atmosphere. The mechanisms evaluated were changes in pectic substances and starch. Results revealed that potatoes with low moisture content (76%) developed significantly ($p \leq 0.05$) more hard tissue than those with higher moisture (80-83%) after 7 d refrigerated storage. The lump formation appeared to be related to a decrease in water-soluble fraction of pectic substances in the cell wall and middle lamella, and to an increase in gelatinization temperature of starch. Low tissue pH caused by antibrowing agents and vacuum packaging, or packaging under the atmosphere of low O_2, appeared to accentuate the lump development. Though there was no difference in Ca^{2+} and Mg^{2+} contents of the cell wall extracted from hard tissue and from fresh tissue, it is possible, nevertheless, that the cell wall and middle lamella in hard tissue were strengthened through Ca^{2+} or Mg^{2+} complexes formed on pectin chains with free divalent ions. This was corroborated by the evidence of dense fibrous material linking adjacent cells in hard tissue, which was not evident in cooked fresh potatoes, as examined under a scanning electron microscope.

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LIST OF ABBREVATIONS

AA:	Ascorbic acid
AIS:	Alcohol insoluble solids
CA:	Citric acid
DE:	Degree of esterification
DSC:	Differential scanning Calorimeter
EDTA:	Ethylenediaminetetraacetic acid
GC:	Gas chromatograph
LDPE:	Low density polyethylene
MAP:	Modified atmosphere packaging
PME:	Pectin methylesterase
PPO:	Polyphenol oxidase
SAPP:	Sodium acid pyrophosphate
SEM:	Scanning electron microscopy
SHMP:	Sodium hexametaphosphate
Tc:	Conclusion temperature
To:	Onset temperature
Tp:	Peak temperature

CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

A. Introduction

Pre-peeled potatoes, including whole and sliced forms are becoming more popular. These products are used in restaurants and other food service establishments. They are also used by producers of French fries and potato chips, and other food processors. Three main benefits to these businesses are convenience, less handling and freshness.

Like other minimally processed fruits and vegetables, pre-peeled potatoes are very perishable and have to be kept refrigerated. When the skin that acts as a protective layer is removed, many problems are created: cell damage during the peeling process, liberation of oxidizing enzymes, and microbial contamination due to the exposure of potato tissue to the environment. Two major problems encountered in pre-peeled potato slices are enzymatic browning and tissue hardening. The latter causes the potatoes to remain hard after cooking, making them unsuitable for consumption.

Tissue hardening is a serious problem facing the pre-peeled potato industry. When it occurs it can result in the formation of large lumps which cannot be mashed after cooking. The causes of the hardening are still not clear. Pre-treatments such as dipping the pre-peeled potatoes in anti-browning solution and enzymatic activities during storage have been suspected.

A compact layer has been observed in potato dices treated with sodium bisulfite, but none was found on samples treated with citric acid (Svensson, 1971). Sapers *et al.* (1995) have found that pre-peeled whole potatoes subjected to digestion with hot ascorbic acid and/or citric acid before dipping in conventional browning inhibitor solution developed surface hardening which became evident after cooking. Surface hardening was found to be more severe when potatoes were dipped in antibrowning solution at elevated temperature (Sapers *et al.*, 1997). It is not clear what causes surface tissue of potatoes to become hard. However, it is suspected that activation of pectin methylesterase followed by cross-linking of liberated carboxyl groups by endogenous calcium ion probably causes this problem. They also suspected that antibrowning chemicals possibly caused this change.

Not much research has been conducted on the tissue hardening problem. A better understanding of the nature of the problem would help find solutions to this phenomenon, which would benefit the pre-peeled potato industry. The main objective of this work, therefore, is to attempt to understand the parameters related to the development of tissue hardening in pre-peeled potatoes and to find possible solutions for this defect.

B. Literature Review

1. Processing of pre-peeled potatoes

Pre-peeled potatoes may be produced in different forms, e.g. peeled and trimmed, raw, whole potatoes; cut forms, such as dices, slices, and French fried strips. As the industry developed, products were preblanched or par-fried to make them even more convenient. These products are commonly used in restaurants and institutional food service markets, providing convenience due to minimum preparation requirements. However, retail packages for household use are not as popular. This kind of potato product is also known as pre-prepared, ready peeled, fresh-peeled, fresh-blanched, etc. (Feinberg *et al.*, 1987; Huxsoll and Bolin, 1989).

The initial processing steps usually include peeling, trimming, and slicing. After these initial steps, potatoes are treated with chemicals for prevention of discoloration. Following the chemical treatment is the cooling of the treated product before packaging, if heat is applied (Feinberg *et al.*, 1987; Huxsoll and Bolin, 1989). The natural skin protects potato flesh from microbial and insect attack, and loss of moisture. Once the skin is removed, the potatoes become very perishable. In order to prolong shelf-life, the storage of pre-peeled potatoes involves a combination of low temperature, a measure to minimize moisture loss and optimization of atmosphere composition (Reyes, 1996). Although pre-peeled potatoes are subjected to only minimal and gentle processing, plant products undergo substantial changes, including alterations of the tissue and cell wall structure during these processes. The changes in tissue structure and properties depend both on internal factors (raw material parameters such as pH and ionic strength) and on external factors (type of processing, external stress etc.) (Kunzek *et al.*, 1999).

1.1 Peeling

Peeling is one of the critical steps affecting changes in tissue. Abrasion and low-temperature lye peeling are the most widely used peeling methods for pre-peeled potatoes. Peeling methods involving the use of heat are not possible for pre-peeled product. At temperature above about 71°C, gelatinization of starch can occur (Harrington *et al.*, 1956). Such a cooked surface presents an unattractive, non-uniform appearance in the cut pieces of potatoes and it tends to become permanently toughened after a few days of holding under refrigeration. Such potatoes are not suitable for mashing because of lump formation in the product. However this problem is highly dependent on cultivar and growing area (Feinberg *et al.*, 1987).

1.2 Cutting

Texture loss and changes in appearance are the most noticeable changes occurring in fruits and vegetables during prolonged storage. These undesirable changes are accentuated by the mechanical rupturing of cells caused by the physical actions of minimal processing, such as peeling and cutting.

The response of plant tissue to mechanical injury or wounding is a complex series of events designed to repair the damage and hence bring about changes in metabolism (Rolle and Chism III, 1987). The main physiological manifestations of this phenomenon include increased respiration rate, stressed ethylene production, cellular disruption leading to closer contact of enzymes and substrates, and wound-healing processes (Rolle and Chism III, 1987; Varoquaux and Wiley, 1994; Brecht, 1995). The consequences of wound-healing are chemical and physical in nature, such as oxidative browning reactions and lipid oxidation. It was found that peeled potatoes have respiration rate two times higher than intact potatoes, while those of sliced potatoes are six times higher than intact ones (Gunes and Lee, 1997).

Mechanical ruptures from peeling or cutting will expose parenchyma cells, containing high water content, sugars, organic acid and other organic substances, and allow these substrates to easily react with active enzymes (Rolle and Chism III, 1987; King and Bolin, 1989; Watada *et al.*, 1990).

Wounding also induces a number of enzymes which contribute directly and indirectly to browning. Phenylalanine ammonia lyase (Hyodo *et al.*, 1978) and polyphenol oxidase (Bower and Van Lelyveld, 1985) were found to be induced by wounding. It is also able to induce lipoxygenase activity which could cause membrane lipid degradation resulting in membrane disruption and production of compounds which reflect flavor of fruits and vegetables (Kalbrener *et al.*, 1974; Brecht, 1995). Lipoxygenase activity is also found to have a bleaching effect on carotenoids (Blain, 1970) and chlorophyll (Holden, 1974).

Wounding may cause water loss from tissues, which adversely affects the texture of produce (Rolle and Chism III, 1987). In whole organs, water in intracellular spaces is not directly exposed to the outside atmosphere. Cutting or peeling a fruit or vegetable exposes interior tissues and drastically increases the water evaporation rate. The difference in the rate of water loss between intact and wounded plant surfaces varies for individual plants. As much as 500-fold difference was found for cut potato tubers (Brecht, 1995).

Changes in tissues of fruits and vegetables can cause loss of firmness through conversion of insoluble protopectin to pectin, decrease in cellulose crystallinity, decrease in galacturonic acid, reduction in cell volume, and thinning of cell walls (King and Bolin, 1989). Enzymes such as β-galactosidase and exo-polygalacturonase initiate most of the undesirable textural changes by hydrolyzing pectic compounds, resulting in loss of cellular turgor (King and Bolin, 1989).

Slicing plant tissue generally results in loss of firmness. Ponting *et al.* (1972) observed loss of firmness with apple slices. Voroquaux *et al.* (1990) found loss of 50% of initial firmness of kiwifruit slices in <2 days at 2°C. They suggested that textural breakdown during storage is due to enzymatic hydrolysis of cell wall components. Pectinolytic and proteolytic enzymes liberated from cell damaged by slicing could diffuse into inner tissue. Watada *et al.* (1990) emphasized the role of ethylene in the loss of firmness of sliced kiwifruits packed together with banana sections. They suggested that ethylene, resulting from wound responses, can increase the permeability of membranes and perhaps reduce phospholipid biosynthesis, which can upset the dynamic process of cellular structure and membrane integrity.

However, in some cases, tougher tissue after storage has been noticed, such as in pre-peeled potatoes. This is probably due to the differences in the components of individual plants. High starch content in potatoes could also contribute, to some degree, to the tissue firmness after prolonged storage.

1.3 Prevention of enzymatic browning discoloration

Enzymatic browning reaction is one of the major problems in pre-peeled potatoes. Previously, sulfites have been used effectively to prevent browning. However, concerns about adverse reactions in the minority of consumers to sulfites in foods has led to the restriction of the use of sulfites and/or the requirement of warning labels on treated food by the FDA (Santerre *et al.*, 1991; Sapers and Miller, 1992; 1993). Various sulfite substitutes, generally a combination of ascorbic acid or erythorbic acid, with such adjuncts as citric acid, calcium salts, sodium acid pyrophosphate or other phosphates, and cysteine, have been used (Duxbury, 1987; 1988; Sapers and Miller, 1992).

Ascorbic acid is normally used for pre-peeled potatoes. Like sodium bisulfites, ascorbic acid acts as a reducing compound which prevents browning by reducing the o-benzoquinones back to o-dihydroxyphenols or by irreversible inactivation of polyphenaloxidase (Whitaker and Lee, 1995). Citric acid, sulfur-containing amino acid such as L-cysteine, calcium salts, sodium acid pyrophosphate (SAPP) or other phosphates, ascorbic acid -2-phosphates, and their combinations have been used for pre-peeled potatoes (Whitaker and Lee, 1995). However, none of them are as effective as sulfites. A combination of chemical treatment and vacuum or modified atmosphere packaging has been reported to be more effective than chemical treatment alone (O'Beirne and Ballantyne, 1987; Giannuzzi and Zaritzky, 1991; Gunes and Lee; 1997). With both chemical treatment and appropriate packaging technique, shelf life of up to 2 week has been observed.

Besides the use of antioxidants, chelating agents and removal of oxygen by packaging techniques for browning inhibition, other methods have also been used. Surface digestion before chemical treatment is an alternative method to control browning in pre-peeled potatoes. Sapers and Miller (1993) digested peeled potatoes with 14-20% NaOH at 35-55°C for 1-13 min. After removal of digested tissue, ascorbic acid-based browning inhibitor was used and shelf life of 13-15 days was obtained.

Vacuum and pressure infiltration has also been used to increase diffusion effectiveness of antibrowning agents. Pressure infiltration at 108 kPa can extend the shelf life of potato plugs by 2-4 days, compared to dipping (Saper *et al.*, 1990).

Sapers and Miller (1995) found that the use of a combination of mild heating and exposure to acids could prevent browning effectively. Peeled potatoes were heated for 5 to 20 min at 45-55°C in 1% ascorbic acid and 2% citric acid, cooled, and then dipped for 5 min in browning inhibitor solution containing ascorbic acid, citric acid and sodium pyrophosphate. By this treatment, shelf-life of 14 days has been achieved with the storage at 4°C.

1.4 Packaging

When sulfite was still commonly used as the antibrowning agent, bag type packages, such as bags with two-ply construction, consisting of an outer Kraft paper with a polyethylene layer, were most generally used for distributing high quantities of product for institutional use. After restriction on the use of various forms of sulfites for preservation, substituting agents have been developed. Modified atmosphere packaging subsequent to antibrowning treatment becomes necessary in order to maintain the acceptable quality (Duxbury, 1987; Feinberg *et al.*, 1987).

Modified and controlled atmospheres by the reduction of O_2 and/or elevation of CO_2 help maintain quality and extend storage life by inhibiting metabolic activity, decay, and, especially, ethylene biosynthesis and action (Kader, 1986). As a result, inhibition of browning discoloration, microbial spoilage and pathogens can be achieved.

Semipermeable plastic films are chosen for MAP so that the film permeability and product respiration can combine to produce a desirable, steady-state atmosphere within the package. Because of the perishability of the product, the MAP atmosphere is often passively established after packaging in the bags. An equilibrium atmosphere of about 5-10% O_2 and 5-10% CO_2 was developed after storage (Reyes, 1996). However, because the establishment of the equilibrium modified atmosphere by respiration may be too slow to prevent discoloration, for a more effective application, the MAP atmosphere is normally effected by flushing with the desired atmosphere or by pulling a slight vacuum and then injecting a desired gas mixture into the package (O'Beirne and Ballantyne, 1987; Reyes, 1996).

It was found that the most effective method of preventing browning and extending shelf-life is obtained from vacuum or oxygen-free atmosphere packaging (Duxbury, 1987; O'Beirne and Ballantyne, 1987). However, the anaerobic conditions prevailing in vacuum packages may result in off-flavor development and would facilitate growth and toxin production by *Clostridium botulinum* at storage temperatures > 4-5°C (Hotchkiss and Banco, 1992). Maintenance of oxygen concentration at low level (slightly above 0) but not over 5 % is commonly used for pre-peeled potatoes storage (Gunes and Lee, 1997). Several factors, including product respiration rate, fill weight, gas permeability of packaging material and surface area of gas exchange, determine the final gas composition inside a sealed package (Tomkins, 1962). Therefore, these factors have to be considered in order to obtain the desired atmospheric condition.

1.5 Storage

Refrigeration is a mandatory preservation method for all minimally processed fruits and vegetables in order to slow down deteriorative physiological processes and microbial spoilage, and reduce the risk from pathogens. Longer shelf life is generally achievable at temperatures close to the freezing point of the product. Storage temperatures between freezing and 4.4°C are recommended (Feinberg *et al.*, 1987; Reyes, 1996). It was suggested that with proper treatment and reasonably sanitary precautions to prevent microbial contamination at the time of processing, peeled potatoes may be held for 5-10 days without spoilage at low temperature.

2. Problems related to pre-peeled potatoes

2.1 Enzymatic browning

Enzymatic browning discoloration is one of the most serious problems in pre-peeled potatoes. As discussed earlier, it is found in peeled, cut or injured raw potatoes as a result of enzymatic oxidation in injured cells when they are exposed for a short time to air or oxygen (Wiley, 1994). This type of browning discoloration is caused by polyphenol oxidase (PPO), a group of enzymes that catalyze the oxidation of phenolic compounds, with the presence of oxygen, to quinones and their subsequent condensation to dark-colored pigments on cut surface of potatoes (Sapers and Miller, 1992; Whitaker and Lee, 1995).

The principles of browning prevention are inhibition/inactivation of the enzyme such as by heating, elimination/transformation of substrate(s), and combinations of above methods (Vamos-Vigyazo, 1995). Lowering the pH by 2 or more below the optimum pH, or removal of one or both of the substrates (O_2 and phenols) can also prevent browning (Whitaker and Lee, 1995).

The enzymatic browning is of commercial importance, primarily to those in the potato prepeeling business. Practical methods to prevent this type of discoloration have been discussed in section 1.3 to 1.5.

2.2 Tissue hardening

Another serious problem encountered in pre-peeled, sliced refrigerated fresh potatoes is the hardening of tissue after prolonged storage. Sapers and Miller (1995) examined ascorbic/citric acid-treated abrasion-peeled potatoes after boiling and found the presence of a layer at the peel surface of several mm thick. A shell-like layer of toughened tissue, which could be separated from the underlying tissue, created an unsightly product and interfered with mashing. They postulated that this problem is due to the activation of pectin methylesterase during heat treatment, especially at 55°C, and the effect of citric acid on pectin degradation.

Many other observations on tissue hardening problems have been reported. Hard tissue formation after browning inhibitor treatment without heating has been observed. A compact layer was found in potato dices (cultivar Bintje) treated with sodium bisulfite but none was found on samples treated with citric acid (Svensson, 1971). Textural differences between potatoes (Russet Burbank) treated with erythorbic acid and/or citric acid and water packaged were observed. The raw potato tissue was found to be less crisp and some differences in mashed potato quality were detected (Santerre *et al.*, 1991). The authors did not elaborate on the differences.

Hard tissue development is also observed in other plants: in chestnuts after preheating (Manabe, 1980), bean seeds stored at high temperature and humidity (Liu *et al.*, 1992), and hardening of Japanese radish after pressure treatment (Kasai *et al.*, 1997). These problems have been connected to the formation of bridge bonds of deesterified pectin and divalent metal ions. Therefore, cross-linking between metal ions and pectic substances, induced by browning inhibitor treatments, can probably cause hard tissue development in pre-peeled potatoes.

3. Pectic substances and potato firmness

3.1 Cell wall and pectic substances

The plant cell wall is the principle structural component of the cell. It has mechanical function as a structural barrier (Kunzek *et al.*, 1999). Cell walls and middle lamellae are major factors contributing to firmness of plant tissue. Change in the composition and structure of the cell wall, as well as the tissue structure, can cause variations in plant texture. The cell wall can be divided into three layers: middle lamella, primary and secondary cell wall. The middle lamella connects single cells to form tissues. The main components of the middle lamella are pectic substances (Van Buren, 1991; Kunzek *et al.*, 1999).

Cell walls can be present as a cellulose-xyloglucan network, or sometimes called cellulose-hemicellulose network, embedded in a pectin matrix. Cell wall strength is established by the cellulose microfibrils, and the pectin matrix acts as a "glue" that holds the microfibrils around one cell and between cells together (Carpita and Gibeaut, 1993; Van Marle *et al.*, 1997). In the cellulose-xyloglucan network, cellulose exists as microfibrils of many thousands of macro molecules (Carpita and Gibeaut, 1993). These microfibrils, consisting of crystalline and para-crystalline structures, are densely packed and stabilized by intra- and intermolecular hydrogen bonds and are nearly water-free (Heredia *et al.*, 1995). Xyloglucans interlock the cellulose framework and are attached by hydrogen bonds. The pectin matrix is an amorphous phase and, in contrast to cellulose network, can take up water easily (Carpita and Gibeaut, 1993; Heredia *et al.*, 1995).

The principle constituent of the pectin polysaccharides is D-galacturonic acid, joined in chains by means of α-(1→4) glycosidic linkages. Inserted into the main uronide chain are rhamnose units, joined to the reducing end of the uronide by (1→2) linkages and the nonreducing end of the next uronide unit by (1→4) bonds. Rhamnose introduces a kink into the otherwise straight chain. Often, arabinan, galactan, or arabinogalactan side-chains are linked (1→4) to the rhamnose (Van Buran, 1991).

An important factor characterizing pectin chains is the degree of esterification (DE) of the uronide carboxyl groups with methyl alcohol. Pectins might be formed initially in a highly esterified form, undergoing some esterification after they have been inserted into the cell wall or middle lamella. There can be a wide range of DEs dependent on species, tissue, and maturity. In general, tissue pectins range from 60 to 90% DE (Van Buren, 1991). In potato, DE could be different depending on the type of pectin extracted. Pectic substances make up about 52-55% of the potato cell wall/middle lamellae (Hoff and Castro, 1969; Jarvis *et al.*, 1981). The high portion of pectic substances in cell wall and middle lamellae emphasizes their importance in potato texture.

Pectins have frequently been classified by the procedures used to extract them from cell walls. In general, three types have been distinguished: water-soluble pectins extractable with water or salt solutions; chelator-soluble pectins extractable with solutions of chelating agents such as ammonium oxalate, ethylenediaminetetraacetic acid (EDTA); and, protopectins which can be dissolved with alkali solutions or hot dilute acids. The level of each fraction is dependent on many factors such as variety, maturity, storage time (Bettelheim and Sterling, 1955).

Protopectin constitutes 69-77% of the total pectic substances in raw potatoes (Talburt *et al.*, 1987; Lisinska and Leszczynski, 1989). It is a highly polymerized form of pectin but exhibits a low methylation rate, while calcium content was found to be highest in this fraction (Bettelheim and Sterling, 1955). It is strongly associated with the cell wall structure. Freshly harvested potatoes are relatively high in protopectin but it will decrease upon storage (Talburt *et al.*, 1987; Lisinska and Leszczynski, 1989).

Soluble pectin contributes about 6-13% of the total anhydrouronide presented in stored potatoes (Talburt *et al.*, 1987). Soluble pectins are of low molecular weight, exhibiting a high methylation degree and containing less calcium than other pectic substances (Lisinska and Leszczynski, 1989). DE of 47-66% was found in this fraction in raw potatoes of different varieties (Bettelheim and Sterling, 1955).

The pectic acid fraction or chelator-soluble pectin accounts for 13-25% of the total anhydrouronide of potatoes. Their calcium and magnesium salts are the main components of intracellular substances cementing the cells and tissues of potato tubers (Talburt *et al.*, 1987; Lisinska and Leszczynski, 1989). DE of this fraction was found to vary from 16 to 39% dependent on potato varieties (Bettelheim and Sterling, 1955).

3.2 Role of pectin on tissue firmness

The middle lamella connects single cells to form tissues. The main components of the middle lamella are pectic substances. So, pectin, in most plant tissues, acts as a cementing substance in the middle lamella (Van Buren, 1991; Kunzek *et al.*, 1999). They are brought into solution more easily than other cell wall polymers and are more chemically reactive. These lead to the frequent observation that the processes that lead to textural changes, such as ripening, storage and cooking are accompanied by significant changes in the characteristics of the pectic substances (Keijbets, 1974; Van Buren, 1979; Van Buren, 1991; Kunzek *et al.*, 1999).

Cooking has major effects on the pectic materials of the potatoes. Upon cooking, middle lamella breaks down or solubilizes as a result of de-polymerization of pectic polymer through β-elimination and breakage of hydrogen bonds, resulting in the loosening of cell wall, and starch gelatinization (Bettelheim and Sterling, 1955; Van Marle *et al.*, 1992; Van Marle *et al.*, 1997; Ng and Waldron, 1997; Binner *et al.*, 2000). These make potato cells easy to separate and softer than raw potatoes. Cell wall yield and uronic acid content were reduced by cooking due to solubilization of pectic polysaccharide. The degree of methylation was also reduced by cooking, and more acetyl group were detected (Van Marle *et al.*, 1997; Binner *et al.*, 2000). There is an increase in the water-soluble pectin fraction, with a decrease in the alkaline-soluble fraction. There was also a decrease in a calcium content of the cooked potatoes as it dissolves into cooking medium (Bettelheim and Sterling, 1955).

Studies show that the changes in the cell wall structure of fruits and vegetables during processing are often caused by the solubilization of pectin. Pectic material is found to be released upon cooking as it is detected in cooking solution (Hughes *et al.*, 1975a; Van Marle *et al.*, 1994). Pectic substances were found to have major effects on textural characteristics of cooked potatoes. Van Marle *et al.* (1992) have observed that mealy potatoes have little intercellular contact and a smoother cell surface than do firm potatoes. Pectic substances in middle lamella in mealy potatoes easily dissolve upon cooking following by cell separation, which result in mealy texture, while in waxy potatoes, cells remain connected together after cooking. It was also found that when mealy potatoes were cooked, they released more pectic material, resulting in more cell sloughing than the non-mealy potatoes (Van Marle *et al.*, 1994).

A change in the composition of potato cell wall material is normally connected with changes in the microstructure and macrostructure. A relationship between texture and pectic substances have been suggested in some studies. Loh *et al.* (1982) found that degradation of middle lamella material was a major cause for loss of fracturability measured by texture profile analysis. When pectic substances, starch and cell size in the starting material have been kept constant, the compressive strength of cooked disks is very closely related to the degradation of the pectic substances as reflected by their release into the cooking liquor (Hughes *et al.*, 1975a). A clear relationship was found between firmness and the amount of pectin. Firmness test using a rheometer showed a higher value with samples containing high level of galacturonic acid. In the same study, they also found that the amount of pectic substance with higher molecular weight (water-insoluble pectin), remaining in the tissue, affected firmness. Sasaki *et al.* (1999) suggested that potatoes with the higher water-insoluble pectin had firmer texture.

These studies revealed the close relationship between pectic substances and potato texture. Any changes in pectic substances could also result in textural changes. Therefore, hard tissue formation may possibly link to changes of pectic substances.

4. Starch and potato firmness

4.1 Potato starch

In potatoes, starch contributes about 65-80% of the dry weight of the tuber (Talburt *et al.*, 1987). Starch is present as microscopic granules in the leucoplasts lining the interior of the walls of the cells of the parenchyma tissue. The distribution of starch within a tuber is tissue dependent. Each potato cell contains starch granules of different sizes. The granule size distribution is also tissue dependent, whereby parenchyma tissue contained larger granules than, for instance, pith tissue. It is not clear if the granule size distribution is cultivar dependent (Burton, 1989).

4.1.1 Chemical nature of potato starch

The two main components of starch, amylose and amylopectin, are present in a ratio of 1:3, which seems to be fairly constant, but does increase with increasing granule size during maturation of the tuber (Talburt *et al.*, 1987). Amylose is a polydisperse polymer of α-1,4- linked glucosyl residues, with slight branching. Amylopectin is a branched-chain glucose polymer in which the α-1,4 linkages are branched by a α-1,6 linkage every 20 glucosyl residues on the average (Talburt *et al.*, 1987).

Negatively charged phosphate groups are present in potato starch. Most of these groups are esterified onto glucose residues in amylopectin (about 1 phosphate group per 300 glucose units) (Swinkels, 1985). This phosphate groups partly accounts for the high degree of swelling of potato starch granules.

4.1.2 Structure of potato starch granule

Wide angle X-ray scattering has shown that the starch granule is a semi-crystalline entity which contain both crystalline and amorphous regions (Banks and Greenwood, 1975). Starch crystallinity is due to the presence of parallel clusters of short chains packed side by side. These chains could be either the external amylopectin chains (the principle crystalline component) or part of the amylose molecules (less important) which can give analogous parallel chain structures by chain folding. Double helical structures have been proposed in both cases (French, 1984; Biliaderis *et al.*, 1980). These crystallites make the starch granules birefringent and have distinct x-ray diffraction patterns. The amorphous regions are those where chain folding or multiple branching (amylose and branching regions or amylopectin) occur and prevents the formation of ordered polymer structures (Biliaderis *et al.*, 1980). The common starch can be classified into three main types with respect to crystalline structure: A-, B- and C- type. The A-type is found in most cereal starches, the B-type in tuber starches and C-type, intermediate between A- and B-type, in bean and root starches. Water molecules are integral parts of the starch polymorphs A and B, the amount of water varying for the different types (Colonna *et al.*, 1987; Keetels, 1995).

4.2 Role of starch on tissue firmness

4.2.1 Starch gelatinization

Upon heating in the presence of water, when the temperature of a potato is raised to above 50°C, water passes from the nonstarchy parts of the cell into the starch granule.

The starch will begin to gelatinize in the range of 64-71°C (Talburt *et al.*, 1987). The starch granule is composed of linear and branched starch molecules associated by hydrogen bonding either directly or through water hydrate bridges to form radially oriented micelles or crystalline areas of various degrees of order. Gelatinazation occurs when an aqueous suspension of starch is subjected to heat, which weakens the micellar network within granules by disrupting hydrogen bonds and allows water molecules to attach to the liberated hydroxyl groups (Leach, 1965; Blanshard and Franks, 1987). The most important physical changes following gelatinization is granule swelling. Cooking time, temperature and relative amount of starch and water present determine the properties of the gelatinized starch (Burton, 1989).

Potato starch exhibits exceptionally high swelling unlike cereal starch. This is partly due to the presence of ionizable esterified phosphate groups (0.06-0.1% as P, dry weight) which assist swelling by reason of mutual electrical repulsion and absence of lipid and protein (Leach, 1965; Galliard and Bowler, 1987). Cereal starches give lower paste viscosities, less clear gel and higher rate of retrogradation due to the absence of chain-repelling phosphate groups, and the presence of amylose-lipid complexes and internal protein (Galliard and Bowler, 1987).

Several factors affect gelatinization characteristics of starch granules. It was suggested that the difference in phosphorous content is responsible for the difference in the gelatinization properties of granules with different sizes. Starch paste prepared from larger potato granules containing lower amount of phosphate was found to have higher viscosity than that prepared from smaller granules (Yamada *et al.*, 1986). Wong and Lelievre (1982) studied swelling capacities of different wheat starches. High swelling capacities are associated with relatively disordered arrangements of polymer within granules. Their study indicated that small granules tend to be more crystalline than large granules, and that small granules of wheat starch have greater swelling capacities than large granules under the condition of paste formation.

The swelling and solubility behavior and gelatinization temperature range of starch is altered radically by chemical modification. It was found that calcium is an important factor deciding properties of potato starch gelatinization (Yamada *et al.*, 1986). Yamada *et al.* (1986) found that gelatinization temperature was raised and viscosity was decreased when potato starch was treated with $CaCl_2$. Gelatinization temperature was decreased when potato starch was subjected to EDTA or citric acid, which act as a chelating agent for calcium. A large drop in both solubility and swelling power was found when hydrogen ions on potato starch phosphates were substituted by monovalent cations, and even more drop was found when Ca^{2+} and Mg^{2+} were used (Haydar *et al.*, 1980). Metal cations would diminish the repulsive force produced by ionized form of starch phosphate. Also, Ca^{2+} and Mg^{2+} would bring about cross-linkages between the phosphates on adjacent chains, thus causing further contraction of swollen gelled starch grains (Chung and Hadziyev, 1980).

Treatment of starch at high temperature (95-110°C) and low moisture contents (18-27%), the so called heat-moisture treatment could induce important structural changes and modifications of gelatinization behavior. Kulp and Lorenz (1981) found that the crystalline structure of potato starch transformed from B-type into a mixture of C-type when it underwent heat-moisture treatment. As a result, higher gelatinization temperature and decreasing swelling power have been observed.

4.2.2. Starch and potato texture

Starch is the major solid component of potato tubers. The amount of starch is generally closely paralleled by the dry matter content of the tuber and by its specific gravity. In potatoes the dry matter content normally varies between 16% and 26% (Burton, 1989). Tubers with high starch content or high dry matter content tend to be mealy in texture after cooking (Linehan and Hughes, 1969; Chung and Hadziyev, 1980). Also, larger tuber cell size and larger starch grains are associated with mealiness (Chung and Hadziyev, 1980). Barmore (1937) found an inverse relationship between the depth of penetration by a penetrometer and the starch content of the tuber; this indicated that tubers with high starch content were significantly firmer than those with low starch content. These findings suggested that the firmness of the cooked tuber might depend on the level of starch present in the tuber.

Texture of cooked potatoes is thought to be controlled by a number of factors, including starch and cell wall component, especially pectic substances (Reeve, 1972; Loh *et al.*, 1982; Javis *et al.*, 1992; Thybo *et al.*, 1998). During heat treatment, two dramatic changes occur: 1). starch gelatinization and swelling and 2). loosening of middle lamella and cell wall swelling (Potter *et al.*, 1959; Hadziyez and Steele, 1979; Keijbets and Pilnik, 1974; Keijbets *et al.*, 1976; Shomer 1995; Shomer *et al.*, 1995). The "swelling pressure" created during starch gelatinization is a major mechanism causing cell rupture and cell separation. The swelling pressure leads to a rounding-off of the cells thereby inducing cell separation. Essential to this concept is the existence of a swelling pressure. This was proposed by several authors, but there was no experimental evidence by measuring cell volumes during heating (Bartolome and Hoff, 1972; Nonaka, 1980).

Recently, Javis *et al.* (1992) measure directly the pressure exerted by starch suspensions (concentrations of about 15% and 40%) during gelatinization. The pressure was in the order of 10^2 kPa and was significantly higher for the more concentrated starch suspensions. Shomer (1995) and Shomer *et al.* (1995) reported an increase in swelling of gelatinized starch with increasing dielectric constant of the solvents. Charged phosphate groups are responsible for this phenomenon by providing the starch polymer with an electrical double layer. Furthermore, they reported that in the presence of excess water, dried cooked potato cells swelled more (about 1.5 times) at 70°C than at ambient temperature.

Any processing that prevents gelatinization of starch could probably result in firmer tissue formation in cooked potatoes. In a study of texture and microstructure of stream cooked, vacuum cooked (VPS-cooked) potatoes, it was found that in the VPS-cooked potatoes the gelatinized starch formed dense clusters. In contrast, water cooked cells were filled with gelatinized starch. The formation of dense clusters of starch affected potato texture by extension of the cooking time in order to get the same softening as water-cooked potatoes. This phenomenon was explained by the high pressure in the pouch which may counteract the starch swelling pressure, and the lack of water which could prevent starch from fully expanding (Thybo *et al.*, 1998).

In conclusion, swelling pressure created by starch gel could be a major factor contributing to potato texture. Any mechanisms causing the reduction of starch gelatinization could result in reduced swelling power and, thus, prevent cell separation. Studies of the changes in the starch in pre-peeled potatoes would be beneficial to the understanding of hard tissue formation.

5. Other factors contributing to potato firmness

5.1 Effects of preheating

When potato tissue is preheated at temperatures between 60 and 70°C then cooled before cooking, the fully cooked tissue is firmer and sloughs less than samples that are boiled without the pretreatment. This phenomenon was found to be associated with the activity of pectin methylesterase (PME) in the tissue, an enzyme that cleaves methyl groups on the polygalacturonic chain (Bartolome and Hoff, 1972). The enzyme is inactive at temperature below 50°C. It is activated above this temperature and reacts with the pectins of the cell wall. The calcium and magnesium contents of cell wall were found to increase due to migration from the gelatinized starch into cell wall at the enzyme effective temperatures. These ions will form metal bridges with free carboxyl groups of the cell wall pectin initiated by PME which will then result in a firmer texture.

On heating, chemical degradation of pectin chain in potato tissue takes place according to the β-eliminative mechanism (besides breaking down of hydrogen bond) which results in the cleavage of the pectin chain next to a methyl-esterified galacturonic acid residue (Keijbets, 1974). The β-eliminative mechanism requires the presence of methyl groups to promote abstraction at C-5 of the uronide residues leading to an elimination at the glycosidic linkage (Sajjaanantakul *et al.*, 1989). Once PME acts on the potato tissue, this polymerization reaction would become less pronounced with decreasing degree of esterification. This could also explain firmer texture associated with preheating.

Therefore, this firming effect of pre-heating process may involve two phenomena. First, the formation of free carboxyl groups increases the possibilities and the strength of calcium and magnesium binding between pectin polymers. Second, the decrease in the methyl ester content would decrease the susceptibility of pectin to the β-elimination chain breaking reaction during cooking (Van Buren, 1979).

However, the role of starch involving increasing firmness after pre-heating have been suggested in some studies. When potatoes have undergone precooking and chilling, marked changes in the physical properties of the gelatinized starch have been described (Potter, 1954; Reeve; 1954). According to Reeve (1954), holding potatoes at 75°C for thirty minutes before cooking resulted in firmer cooked potatoes and no sloughing occurred even after prolonged cooking. Potter *et al.* (1959) observed firming after preheating potatoes at 60 to 82°C at different ranges of time. Reeve (1954; 1967) and Potter *et al.* (1959) theorized that the firming effect was due to starch retrogradation during preheating. Retrogradation decreased the swelling capacity of the starch granules, which will result in less "rounding off" of cell walls, rupture of the middle lamella, and separation of adjacent cells. In his studies, Potter (1954) found that retrogradation of gelatinized potato starch was much more pronounced at 5°C than at higher temperature, including 55°C.

Relationship between amylose and firming texture was also described. Linehan and Hughes (1969) suggested that starch leakage tends to strengthen the intracellular cement. They postulated that during pre-heating when some starch is gelatinized, amylose leaks into the middle lamella and strengthens it by forming hydrogen bonds with other polysaccharides. It is held that the cell wall is sufficiently porous to allow the diffusion of amylose molecules through unbroken cell walls. They attribute the intercellular adhesion, as part of a firming effect, primarily to the levels of amylose and polyunronide, and to a lesser extent to the level of Ca^{2+} and Mg^{2+}. However, amylose content in middle lamella has not been detected to prove this theory.

In one study, it was found that starch but not PME played a role in firmness affected by precooking. Moledina *et al.* (1981) observed that when potato underwent pre-cooking, following by cooling and then cooking, the primary benefit of pre-cooking followed by cooling is not the activation of PME but, rather, a supply of Ca^{2+} by starch gelatinization and stabilization of Ca-bridges within the cell wall pectin. In this study, they found that pre-cooking at the temperature optimal for PME resulted in less tissue firming than higher temperatures where the enzyme is largely inactivated (65 against 75°C). They also found that Ca^{2+} availability is more important than the production of COO^- from PME activity in firmness as measured with penetration force. In order to investigate the effect of Ca^{2+} and firmness, cell wall pectin solubilization in pre-cooking as affected by Ca^{2+} was tested with H-cell walls. In this walls the metal cations bound to free COO^- groups of pectin were replaced by H^+ -ion. Starch phosphate neutralized with Ca^{2+} and Mg^{2+} was also used in this study. They found that Ca-uptake by cell wall could be in excess of the amount of available COO^- groups, which mean that extra Ca^{2+} may be uptaken by phosphoric acid of starch amylopectin left enmeshed in the cell wall and/or of phytic acid adhering to the cell wall. Moreover, when Ca^{2+} is available in starch and potato was precooked at 70°C, pectin is less soluble. This was because Ca^{2+} must have been transferred to pectin- COO^- groups readily from fully gelatinized starch at 70°C. This effect was not found in experiment with Mg-starch, which corroborated the finding of Keijbets *et al.*, (1976) but disagreed with that of Bartolome and Hoff (1972) that implicated both cations in metal bridge formation with de-esterified pectin. This finding could emphasize the effect of starch during pre-heating process.

Saper *et al.* (1995; 1997) suspected that PME could cause surface hardening in pre-peeled potatoes that underwent heating in enzyme inhibitor solution and that involved digestion with hot acid in order to remove damage cells from peeling surface. However, surface hardening of pre-peeled potatoes was also observed in peeled potatoes that did not undergo heating process before packaging. Therefore, if PME is a major factor causing surface hardening, preheating may not be the only condition that activates PME. Peeling and cutting processes that damage potato tissues, antibrowning reagent, or prolonged storage at low temperature may also have some effects on PME activity.

5.2 Effects of pH

Pectic substances can undergo changes when foods are subjected to different conditions. Demethoxylation of the esterified carboxyl group proceeds under mild alkaline conditions or in mild acid (Van Buren, 1979). Besides elevated temperature, weakly acidic condition is found to enhance pectin breakdown by β-eliminative mechanism, whereas lowering the pH had a very marked effect on increasing intercellular adhesion (Hughes *et al.*, 1975b). The higher the pH (above 5), the weaker is the hydrogen-bonded pectin gel, probably due to polyuronide hydrolysis via β-elimination. At pH value below 3 the pectin gel is again weakened due to acid hydrolysis of glycosidic bonds but most difficult to degrade at about pH 3.3-4.5 (Albersheim *et al.*, 1960; Doesburg, 1961; Sterling, 1968; Fuchigami, 1983a; Fuchigami, 1983b; Sasaki *et* *al.*, 1999). Wager (1963) using a taste panel, found a very marked effect of pH on texture of potatoes tuber. Softening of potato tissue was found at pH 6.

Sajjanantakul *et al.* (1989) found that the β-elimination is responsible for the heat degradation of cell wall or middle lamella pectin under pH 5.0 to 6.5, conditions found in most processed vegetables. The high methyl ester content of pectin results in more pectin degradation. Depolymerization of pectin at alkaline condition and at pH 6.8 have been suggested by several authors (Albersheim *et al.*, 1960; Whistler and BeMiller, 1960). It was found that pectin with high DE gave lower pH in cooking solution. This was caused by partial de-esterification resulting in more carboxylic acid group being exposed. Although, high DE content enhances the pectin breakage, it also results in lowering pH of solution after cooking, which in turn interferes with the β-elimination. Therefore, at high DE solution, the amount of eliminative cleavage was only less than 2% of total glycosidic bonds. This study could explain the crucial influence of pH on pectin degradation.

Hughes *et al.* (1975b) have observed increasing of acidity in cooking liquor when $CaCl_2$ was used for cooking potato disks. This increase resulted from the binding of calcium by potato tissue, which would increase the level of hydrogen ions in the liquor. They found a remarkable effect of low pH on the increase of tissue firmness. They suggested that this effect was due to Ca^{2+}. When high level of Ca^{2+} was used at pH 6, loss of compressive strength was observed. Similar result was also found on carrots when they were cooked in $CaCl_2$ solution (Sterling, 1968). Sasaki *et al.* (1999) found that effect of pH on firming was stronger than the effect of aluminum ion when sweet potatoes were cooked in a solution. These finding indicated the important effect of low pH on the strengthening of tissue.

The influence of the gradual reduction of pH within carrot tissue from 6.2 to 3.9 by acetic acid infiltration was studied. Maximum firmness was observed at pH 4.4. Total pectin decreased to a less extent at pH 4.4 than at either higher (6.2) or lower (3.9) pH values (Ben-Shalom *et al.*, 1992).

In sweet potatoes, the softening of a French fry-type product decreased linearly when the tissue pH was incrementally lowered from about 6 to 3.8 prior to blanching. Also, the amount of water-soluble pectin decreased linearly as pH decreased and tissue firmness tended to increase as the water soluble pectin content decreased. H^+ ion was primarily responsible for the firming effect. It was suggested that the structure of pectic substances was affected by the tissue pH. Starch hydrolysis also decreased as tissue pH decreased from its normal value of about 6, reflecting partial inactivation of endogenous amylolytic enzymes (Walter *et al.*, 1992).

Plant tissues treated with very high pH also resulted in firmer texture. Beans and carrots treated at pH 12.5 directly after blanching were firmer than samples without alkaline treatment. Same effects were also found in potatoes, carrots, cauliflower and apples (Heli and McCarthy, 1989; Van Buren and Pitifer, 1992; Walter *et al.*, 1992).

Alkaline treatment applied before cooking can change cell wall component by lowering pectin DE, which will suppress pectin degradation by β-elimination (Van Buren and Pitifer, 1992). So, cell wall will not easily breakdown. When soaking samples in EDTA solution after alkaline treatment, samples were less firm. This proves that it is calcium-pectin interaction that causes firmer texture (Van Buren and Pitifer, 1992). However, they did not find this effect of EDTA in beets and dry beans, probably due to their difference in cell wall component and structure.

Pre-peeled potatoes are usually treated with enzyme inhibitors most of which are acidic solutions. From relationship between pH and firmness mentioned above, it is possible that some enzyme inhibitors with high acidic condition may cause tissue firmness or hardening.

5.3 Effects of ions

It has been known that ions affect texture by their interaction with pectic substances. Monovalent ions can reduce firmness and divalent ions increase firmness. Divalent ions can cross-link with molecules of pectinic acid, inhibiting pectin solubilization. Pectate generally forms calcium gels in which the junction zones are composed of unbranched, non-esterified galacturonan chains, linked together by non-covalently bonded calcium ions. The parts of the pectin chains with the higher degree of esterification and containing more side chains are supposed to be more flexible and less susceptible to aggregation (Jarvis, 1984; Van Marle *et al.*, 1994).

Both sodium and potassium appear to reduce texture firmness to a similar degree, probably by displacing the calcium that forms complexes with polygalacturonan (Davis and Tourneau, 1967; Hughes *et al.*, 1975b). Calcium, copper and iron inhibited solubilization of pectic polysaccharides when they are added at the ratios of cation/pectin carboxylic group lower than 1 (Keijbets *et al.*, 1976). In the presence of an excess of divalent cations, or the presence of monovalent ions, the probability of complex formation between cations and pectin carboxylic groups is lower. Therefore, calcium added at the ratios of cation/pectin carboxylic group higher than 1 increased the solubilization of pectic polysaccharides (Keijbets *et al.*, 1976). Calcium or magnesium ion is found to strengthen plant tissue through cross-linking of molecules of pectinic acid to form a more rigid matrix in the middle lamella and primary cell well (Sterling, 1968). However, most studies (Keijbets *et al.*, 1976; Haydar *et al.*, 1980; Moledina *et al.*, 1981) found that magnesium was not able to inhibit solubilization of pectic polysaccharides.

Organic acids in the tissue are present in the anionic form (citrate^{2-}, malate^{2-}, phytate^{12-}) and can enhance solubilization of pectic polysaccharides (Keijbets *et al.*, 1976). These ions have the ability to form complex with calcium, which can result in competing with pectic substances for calcium (Hoff, 1972; Keijbets *et al.*, 1976). When present, they have the effect of reducing the amount of calcium available for interaction with the cell wall constituents, resulting in weakening of middle lamella and an increased tendency to develop cell separation during cooking. It was found that mealy potatoes tend to contain relatively high concentration of these acids (Schwartz *et al.*, 1961).

Treatment with calcium increases firmness in many plant-based products. Firmness of canned sweet potatoes has been increased by treatment with $CaCl_2$ as a soaking solution (Walter Jr. *et al.*, 1992). In Japanese radish roots, it was found that while monovalent cations such as K^+ and Na^+ accelerated pectin solubilization, resulting in softening of vegetables, $CaCl_2$ and $AlCl_3$ inhibited pectin solubilization and prevent tissue softening (Sasaki *et al.*, 1999).

When potato slices were soaked in a solution containing metal such as Al^{3+}, firmer tissue was observed after cooking compared with untreated samples (Sasaki *et al.*, 1999). This suggested that ion from soaking solutions could diffuse into the slices during soaking and has an effect on texture. Enzyme inhibiting solutions used in pre-peeled potatoes is normally in acidic condition. Hydrogen ion from browning inhibitor solution could diffuse into the tissue and possibly cause solubilization and redistribution of Ca^{2+} and Mg^{2+} ion inside potatoes tissue, leading to a firmer texture. Therefore, ions transferring could be an interesting aspect causing the defective texture in pre-peeled potatoes.

5.4 Effects of packaging and storage

During cool storage of minimally processed plants, all enzymes present are still active. Plants will use the endogenous nutrients stored in their tissue as the sources of energy for sustaining their lives. Aerobic glycolysis is the principal process of degradation, in which carbohydrates or other constituents are converted to smaller molecules and energy. As a result, aging or senescence is accomplished with the loss of aromas, flavors (e.g. sweetness), and nutrients. Aerobic glycolysis also results in the accumulation of carbon dioxide and other products such as acids and alcohols, which give off-flavors. When the concentration of oxygen is too low, anaerobic glycolysis occurs and results in the accumulation of acids, aldehydes, ketones and alcohols. These products lead to tissue damage, loss of nutrients and off-flavor development (Labuza and Breene, 1989).

Modified atmosphere (MA) in packages, by lowering O_2 level or increasing CO_2 level, has been found to prolong shelf-life of pre-peeled potatoes effectively (O'Beirne and Ballantyne, 1987; Gunes and Lee, 1997). MA can reduce the incidence of physiological disorder by lowering the metabolism and decreasing O_2 consumption and CO_2 production, microbiological spoilage, and biochemical deterioration (Varoquaux and Wiley, 1994).

High CO_2 levels decrease energy supplied to tissues by inhibiting various respiratory enzymes and uncoupling oxidative phosphorylation (Rolle and Chism III, 1987).

Storage of fruits and vegetables under controlled atmosphere conditions has differential effects on various constituents. Low O_2 levels have been shown to delay chlorophyll loss in tomatoes (Goodenough and Thomas, 1980) and apples (Knee, 1980), and retard the rate of flesh softening in apples (Knee, 1980). Elevated CO_2 levels delay brown discoloration of lettuce (Siriphanich and Kader, 1985) and green beans (Buescher and Henderson, 1977) by inhibiting the formation of phenolic compounds and phenolase activity.

Elevated CO_2 levels can also affect texture. Toughening of asparagus spears (Lipton, 1975) and tenderization of broccoli (Lipton and Harris, 1974) could be retarded by elevated CO_2 levels.

Pectin changes were found to correlate with changes in storage atmospheres. In "Yellow Newton" apples, the increase in soluble pectins is retarded by CO_2 storage (Salunkhe *et al.*, 1991). The CO_2 storage may have an effect on protopectin hydrolysis. The soluble pectin content is much higher where higher temperatures or no CO_2 are involved. Controlled atmosphere-stored apricots and peaches retain a higher concentration of total pectins than when fruits are stored in conventional refrigerators (Salunkhe *et al.*, 1991).

Under MAP and prolonged storage, peeled potatoes still undergo both physical and chemical changes inside their tissues. Injury resulted from the processes, such as cutting or peeling, could accelerate these changes. Therefore, unexpected results may be detected in the products during storage.

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CHAPTER 2. FACTORS AFFECTING THE FORMATION OF HARDENED TISSUE

2.1. Introduction

Pre-peeled potatoes undergo several processing steps, which could have some effects on hard tissue formation during storage. Chemicals are usually applied in order to prevent enzymatic browning discoloration and they are thought to contribute to the tissue hardening problem (Svensson, 1971; Sapers and Miller, 1995). Modified atmosphere packaging in addition to antibrowning treatment was found to be more effective in prolonging the shelf life of minimally processed produce than antibrowning treatment alone (Gunes and Lee, 1997). However, the effects of packaging on hard tissue formation have never been investigated. Besides chemical treatment and packaging, the variety of potatoes can also play an important role in causing tissue hardening due to their different compositions. In preliminary experiments, it was observed that potatoes with different moisture contents gave different amounts of lump tissue after storage. In this experiment, three factors, including antibrowning chemicals, packaging conditions and different types of potatoes were investigated for their effects on hard tissue formation.

2.2. Materials and methods

2.2.1. Effect of various types of enzyme inhibitors on hard tissue formation

Russet potatoes (24-25% dry matter) used for this experiment were Alberta grown, purchased from a local store and held at 4°C until needed.

Prior to peeling, potatoes were washed and sanitized by dipping in 100 ppm sodium hypochlorite solution (Colgate-Palmolive Canada Inc., Toronto, ON) for 1 min, and rinsed briefly with water. Washed potatoes were peeled by hand with a potato peeler. Following the peeling, potatoes were briefly submerged in cold water, cut into French-fry shape with a cross-section of 1x1 cm by a Hobart slicer (Hobart Canada Inc., ON) and submerged in distilled water until browning inhibitor treatment could be applied.

Strips from several tubers were mixed together before being divided into 7 samples of 600 g each and then were treated with different enzyme inhibitor solutions. Enzyme inhibitors used in this study were:

1) 1% citric acid (Sigma Chemical Co., MO, USA)

2) 2% ascorbic acid (Sigma Chemical Co., MO, USA)

3) 2% citric acid and 1% ascorbic acid

4) 1% citric acid and 500 ppm EDTA (Fisher Scientific, NJ, USA)

5) 2% ascorbic acid and 500 ppm EDTA

6) 2% citric acid and 1% ascorbic acid and 500 ppm EDTA

All dipping solutions were prepared with MilliQ water to minimize the effects of interfering ions present in tap water. Potato strips were submerged in the browning inhibitor solution for 10 min followed by draining for 10 min. A ratio of 1:2 of strips to dipping solution was used in every experiment. After draining, the strips were packaged in 2 mil low density polyethylene (LDPE) bags (Super Poly Ltd., AB) and stored at refrigerated temperature. After 1 wk of storage at 4°C, samples were steam-cooked in an Atmosphere Steam Cooker with a covered lid for 40 min before being immediately mashed in a Kitchen Aid Mixer equipped with a flat beater (The Hobart Mfg. Co., Troy, OH) with a setting of 5-6 on the speed control for 1.5 min. Two replicates were prepared for each treatment. As a control, strips were prepared by the same procedure but they were dipped in distilled water for 10 min instead of enzyme inhibitor solution. The following factors were determined in each treatment:

a) pH of dipping solution, and of raw potatoes after dipping, before and after storage

b) amount of hard lumps after cooking and mashing

2.2.1.1 Determination of tissue pH

A modified method of Walter *et al.* (1993) was used. A few strips of treated-raw potatoes were taken randomly from each sample. They were cut into small pieces. A duplicate of at least 15 g of cut potato were a blended in blender (Waring model 31BL92, CT, USA) with twice as much water by weight and the pH of the mixture was measured with a pH meter (Orion model 701A, Orion Research Inc., Mass., USA).

2.2.1.2 Determination of amount of hard lumps

The method of Sapers *et al.* (1997) was modified. A 100 g of mashed potato was suspended in 500 mL of 50% ethanol, stirred vigorously for 1 min, and poured through a No.10 stainless steel standard sieve (opening 2 mm, The W.S. Tyler Co. of Canada, Ltd., ON) to isolate lumps and unmeshed tissue. The collected lumps were washed with deionized water several times to remove adhering small potato particles. The sieve was drained for about 5 min, blotted dry, weighed, and the percentage of lumps not passing through the sieve was calculated.

2.2.2. Effect of potato moisture content on hard tissue formation

Six different types of potatoes were used in this experiment:

- Washington potatoes (Wahluke Produce Inc., Mattawa, WA, USA)
- Yukon gold potatoes (Baker Produce South Inc., Edison, CA, USA)
- Table potato (Westfair Food Ltd., Calgary)
- Table potato (purchased from a local store)
- Kenebec (purchased from a local store)
- Russet potatoes (Morinville Growers RR#2, Morinville, AB)

They were purchased from a local store and held at 4°C until used.

Prior to peeling, potatoes were washed and sanitized by dipping in 100 ppm sodium hypochlorite solution for 1 min, and rinsed briefly with water. Washed potatoes were peeled by hand with a potato peeler. Following the peeling, potatoes were briefly submerged in cold water, cut into French-fry shape with a cross-section of 1x1 cm with a Hobart slicer and submerged in distilled water until browning inhibitor treatment could be applied.

For each type of potatoes, strips from several tubers were mixed together before being divided into samples of 600 g and treated with a solution of 1% ascorbic acid, 2% citric acid and 500 ppm EDTA. This solution was selected from the six enzyme inhibitor solutions because the results from the previous experiment (2.2.1) showed that the treatment produced the highest amount of hard lumps. Each treatment was done in duplicate. After 7 d of storage at 4°C, samples were steam-cooked and mashed.

2.2.2.1. Determination of tissue pH and amount of hard tissue

Tissue pH of raw samples and the amount of hard tissue (lumps) of cooked mashed potatoes were compared among each type of potatoes according to the methods described in section 2.2.1.1 and 2.2.1.2, respectively, on day 0 and day 7.

2.2.2.2. Moisture content determination

The moisture content of each type of potato was also measured. A few slices from each sample were selected randomly and chopped into small pieces. Their moisture content was determined using the hot air oven method. A 4-5 g of chopped sample was placed in a pre-dried and weighed aluminum dish and incubated in the hot air oven at 105°C. After 5 hours, samples were cooled in a desicator and weighed. This procedure was repeated until constant weight was obtained. The moisture content was calculated as a percentage of initial weight.

2.2.3. Effect of packaging conditions and storage time

The russet potatoes (24-25% dry matter) used were Alberta grown (Morinville Growers RR#2, Morinville, AB), purchased from a local store and held at 4°C until used. Russet potatoes were selected for this study because the results in section 2.2.2 indicated that they developed the highest amount of hard tissue.

Prior to peeling, potatoes were washed and sanitized by dipping in 100 ppm sodium hypochlorite solution for 1 minute, and rinsed briefly with water. Washed potatoes were manually peeled with a potato peeler. Following the peeling, potatoes were briefly submerged in cold water, cut into French-fry shape with a cross-section of 1x1 cm with a Hobart slicer and submerged in distilled water until browning inhibitor treatment could be applied.

Strips randomly picked from several tubers were mixed together before being divided into samples of 600 g each and treated with a solution of 1% ascorbic acid, 2% citric acid and 500 ppm EDTA. After draining, sampled were packaged (500 g of strips per bag) in vacuum type plastic bags (2.5 mil Nylon/Polyethylene bags). Four different packaging conditions were used: air packaging, two modified atmosphere packaging of $20\%CO_2+5\%O_2+75\%N_2$ and $20\%CO_2+80\%N_2$, and vacuum packaging. Carbon dioxide (99.8% pure), nitrogen (99.95% pure), and oxygen (99.6% pure) was supplied by Praxair, AB, Canada. In air-packaged samples, the strips were placed in the bag and the bag was heat sealed. Gas mixtures of 20% CO_2, 5% O_2 and 75% N_2 and $20\%CO_2+80\%N_2$ were made by mixing the pure gases in the predetermined proportions with a proportional tri-gas blender (model 180SCFH, Smith Equipment, SD, USA) before flushing the sample bags in a vacuum/gas packaging equipment (Multivac type AG500, Sepp Haggenmuller, KG, Germany). In vacuum packaged samples, air was drawn from the bag before being sealed with the same packaging equipment. All samples were stored in a cooler at 4°C until analyses. Every 2 d, treated samples were monitored for gas composition and raw tissue pH. Color and amount of lumps were also determined after samples were steam-cooked and mashed.

2.2.3.1. Determination of tissue pH and amount of hard tissue

Tissue pH of raw samples and the amount of hard tissue of cooked mashed potatoes were compared for each type of potatoes according to the methods described in section 2.2.1.1 and 2.2.1.2.

2.2.3.2. Color determination

Color was measured with a HunterLab colorimeter (Hunter Associates Laboratory INC., VA, USA). A mashed potato sample of 45 g was placed on a glass Petri dish (diameter of 7.5 cm) and then pressed uniformly to obtain a continuous surface. Hunter L, a* and b* values were measured twice after rotating the container 90^0. Two samples from each treatment were measured. The values were compared with those of freshly mashed sample.

2.2.3.3. Head space determination

Headspace gas analysis was carried out using a gas chromatograph (GC), model GC8700 (Carle) equipped with a thermal conductivity detector. The GC columns were a Porapak A column to separate CO_2 from the other gases, and a Molecular sieve 5A column, to separate O_2 and N_2 (Supelco, ON). Helium was used as a carrier gas at a flow rate of 40 mL/min. The GC was connected to a computer, which recorded the gas mixtures as percentages. A gas sample (5 mL), obtained with a gas-tight syringe from sample bags, was injected into the GC for the analysis. The O_2, CO_2 and N_2 concentrations were calculated as volume percentages of the total gas inside the package.

2.3. Results and Discussion

2.3.1. Effects of various types of enzyme inhibitors on hard tissue formation

All antibrowning solutions had pH within the range of 2 to 3. Solutions prepared from citric acid had somewhat lower pH than those prepared from ascorbic acid (AA). When both acids were used together, the solution pH dropped about 0.6 point comparing with when ascorbic acid was used alone, and about 0.3 point when citric acid was used alone. This was expected due to the higher concentration of hydrogen ion present in the solution. Table 2.1 shows dipping solution pH and tissue pH of treated potato strips at days 0 and 7 and lump percentage of cooked and mashed strips after 7 d of storage.

After dipping potato strips in antibrowning solution for 10 min, there was a decrease in tissue pH by about 1 point, comparing with the control sample of freshly prepared strips with a pH of about 6. Samples treated with the solutions with lower pH resulted in a lower tissue pH. The lowest tissue pH was found on samples treated with solutions containing both CA and AA, while those treated with CA had lower tissue pH than those treated with AA solution ($p<0.05$). However, after storage for 7 d at refrigerated temperature, the tissue pH of all treated samples except in those treated with 2%CA+1%AA+500 ppm EDTA, which remained the lowest at 5.95, increased to a value close to that of freshly prepared samples. The buffering capacity of potato tissues might be responsible for bringing the pH back to the normal level. It was interesting to note that the strips with the lowest pH had the highest amount of lumps, though the relationship between the tissue pH and the amount of lump was much more complex, as shown in Figure 1 (plot of lump vs pH).

Table 2.1. Effect of browning inhibitor treatment on hard tissue formation

Enzyme inhibitor solution	Solution pH*	Tissue pH day 0*	Tissue pH day7*	Lump percentage *#
1. 2%citric acid(CA)	2.61 ^{ns}	5.45^{a}	6.14^{abcd}	5.55^{a}
2. 1%ascorbic acid(AA)	2.96 ^{ns}	5.95^{b}	6.22^{bc}	7.05^{ab}
3. 2%CA+1%AA	2.33 ^{ns}	5.28^{c}	6.15^{abcd}	14^{c}
4. 2%CA+500ppmEDTA	2.65 ^{ns}	5.39^{a}	6.22^{abcd}	8.8^{b}
5. 1%AA+500ppmEDTA	2.97 ^{ns}	6.08^{bd}	6.28^{bc}	13.75^{c}
6. 2%CA+1AA+500ppmEDTA	2.37 ^{ns}	5.15^{c}	5.95^{d}	33.85^{d}
7. UNTREATED**		6.14^{d}	6.14^{abcd}	19.2^{c}

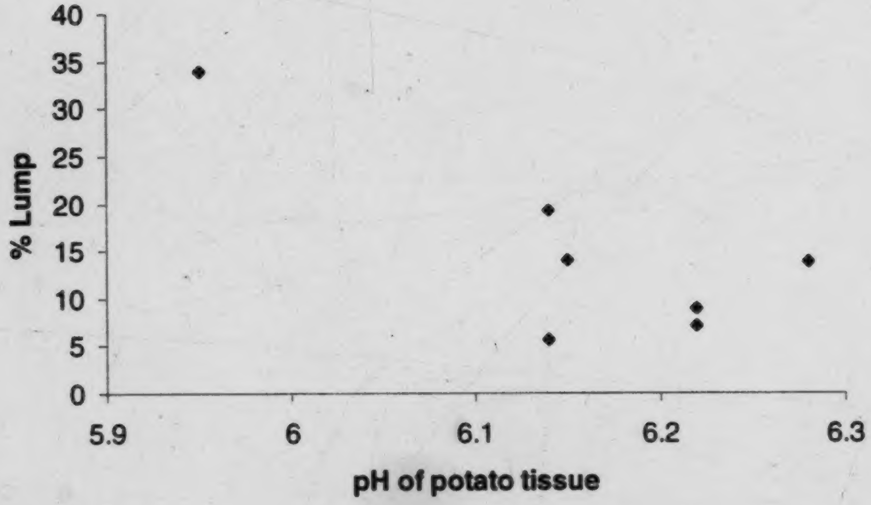
* Data are means of two replicates
** Potato strips dipped in distilled water for 10 min, drained and packaged
# Lump not passing through No. 10 standard sieve
a,b,c,d Mean scores in the same column not sharing the same superscript are significantly different ($p<0.05$)

Different amounts of lump were found in strips treated with different enzyme inhibiting agents. When CA and AA were used, lump percentage was found to be higher than when each was used alone. It was found that when EDTA was added in the dipping solution, higher amounts of hard tissues were found. The size of hard tissues and lumps varied. In samples containing high amount of lumps such as untreated and AA/CA/EDTA, lumps with diameters of about 1 cm or larger were commonly observed, in combination with those of smaller sizes. In samples with a lower amount of hard tissues, only small size (approximately 2 mm diameter) lumps were found. In these cases, new periderms (brown tissues observed on the strip surface), formed during storage, were also retained on the screen during the lump separation procedure. This type of tissue should not be considered as hard tissues or lumps. Therefore, actual lump percentage, in these cases, could be somewhat lower than reported.

There did not appear to be a direct relationship between dipping solution pH or tissue pH and the amounts of hard tissues (Figure 2.1). Moreover, in control samples (untreated samples), hard tissues were also found in quite a high proportion.

Browning discoloration was observed on treated strip surfaces after a few days of storage at 4°C, and only after 1 day of storage for untreated samples. Brown color was apparent on the slice surface and was more pronounced on the peeled sides. This thin brown surface film was not considered as lumps formed on cooking. It was probably formed by surface cells as part of the wound healing process. During peeling these cells were compressed, and formed a thin film surrounding the tubers (Svensson, 1971). Besides brown color formation on raw strips, formation of white material on the surface

Figure 2.1. Relationship between tissue pH and the amount of lump formed



of peeled potatoes was also observed during storage and was quite clearly seen on untreated samples. This whitish appearance or "white blush" has been attributed to lignin (suberin) formation, and dehydration following the peeling and it was reported to be similar to the formation of suberin during wound healing in potatoes (Reyes, 1996).

Chemicals applied to prevent browning discoloration acted differently on potato tissues and could have different effects on hard tissue formation. Citric acid acts in two ways as a browning inhibitor: it lowers the medium pH away from the optimum enzyme pH (5.6-6.7) and it chelates copper in the active site of polyphenol oxidases, thus inactivating the enzymes (Gunes and Lee, 1997). Ascorbic acid is an effective reducing agent. It reduces the quinones formed by polyphenol oxidase action to dihydroxyquinones and it also captures oxygen, necessary for browning reaction. EDTA is a metal-chelating agent that could react with phenolase, a metalloprotein with copper as its prosthetic group, thus prevent browning (Wiley, 1994). However, antibrowning treatments used in this experiment did not appear to be effective in returning a fresh appearance in the stored strips. This is probably due to several reasons; for example, the lack of an effective use of complementary packaging techniques, and insufficient time for antibrowning solution to diffuse into the tissues.

Diffusion of each antibrowning solution into potato tissue, which is time and temperature dependent, could affect the formation of hard tissues. Treatment with AA resulted in more hard tissue formation, probably due to the higher concentration of the acid use and its higher diffusion ability. When diffusion of CA and AA was studied in pre-peeled potatoes, it was found that the effective diffusion coefficients were higher for AA than for that of CA (Giannuzzi *et al.*, 1995; Lombardi and Zaritzky, 1996). When both acids were combined, more severe effects on hard tissue formation could be expected because of the higher concentration of the acid diffusing into the tissues.

It has been suggested that different acids or chemicals used as antibrowning agents can affect the amounts of hard tissue formation after storage. Svensson (1971) found that treatment of peeled potatoes with a solution of sodium bisulfite promoted the formation of the compact layer or hard tissues, while treatment with citric acid did not show this effect. Both sodium bisulfite and ascorbic acid are antioxidants and act similarly as enzyme inhibiting agents, although AA is less effective. In the present experiment, higher hard tissue formation was found in AA treated samples than in CA treated samples. AA or bisulfite may have an effect on other enzymes besides the enzymes causing browning. During boiling of the potatoes, pectinases and cellulases normally affect the middle lamella and the cell wall, respectively. If these enzymes were inhibited by AA or sulfite, it might have some effect on potato texture.

EDTA is a strong chelating agent and can bind calcium and magnesium, resulting in the reduction of intercellular adhesion of cell wall components. EDTA (0.01M) reduced intercellular adhesion of potato tuber sections by 50% comparing with untreated samples. The optimum pH for EDTA for the reduction of firmness at 35°C was found to be between 6.0-7.0 (Lineham and Hughes, 1969c). A low concentration of EDTA, the application of EDTA in acid solutions (pH 2.0 to 3.0), and a short dipping time can explain the lack of reduction in divalent ion binding effects and, hence, hard tissue formation might be expected when EDTA was used. The higher amounts of hard tissue found when EDTA was used may also result from the effect of EDTA on potato enzymes.

In general, various antibrowning agents had different effects on hard tissue formation. However, because untreated samples also developed noticeable amounts of hard tissues, other factors, besides antibrowning agents, could have contributed to this problem as well.

2.3.2. Potato moisture content and hard tissue formation

Tissue pH at days 0 and 7 of treated potato strips of different moisture contents and lump percentage of the strips after 7 d of storage are shown in Table 2.2. The moisture content of potatoes used in this experiment varied from 75% to 86%; i.e., dry matter content varied from 25 to 14%.

After potato strips prepared from potatoes with various moisture content were treated with the solution of 2%CA + 1%AA + 500 ppm EDTA, and stored at refrigerated temperature for 7 d, different amount of lumps were formed. The amounts of lump between samples were significantly different ($p<0.05$). Lump percentage was twice as much when potatoes with lower moisture content (76%) were used, while potatoes with moisture content between 80 to 83% gave similar amounts of lump.

In Kenebec potatoes, which contain 86% moisture, no lump tissue was found after mashing, which resulted in a very soft texture. Their texture after cooking and mashing was different from those prepared from potatoes with a lower moisture, which had a mealy texture and showed more cell sloughing. A thin surface film developed during storage, instead of lump tissue, was the only tissue collected on the screen. This contributed to the high weight of tissue remaining on the screen, but should not be

Table 2.2. Effect of tuber moisture content on hard tissue formation

Potato Sample	Moisture content*	Tissue pH (day 7)*	Lump percentage (day 7)*
1. Table potatoes (Alberta)	75.42 ± 0.98	5.13 ± 0.05	11.85 ± 1.9^{a}
2. Table potatoes	80.32 ± 0.19	5.23 ± 0.11	6.6 ± 1.27^{b}
3. Table potatoes (Washington)	81.03 ± 0.84	5.16 ± 0.14	5.75 ± 0.14^{b}
4. Baking potatoes	81.11 ± 0.05	5.09 ± 0.19	5.65 ± 0.07^{b}
5. Yukon gold	82.09 ± 0.21	4.95 ± 0.06	5.6 ± 1.12^{b}
6. Kenebec	86.45 ± 0.28	4.85 ± 0.18	$16.95\pm2.19^{c@}$

Results are average of two replicates

a,b,c Mean scores in the same column not sharing the same superscript are significantly different ($p<0.05$)

@ Surface film only, no lump

considered as hard lump. Other potato samples also formed small amount of surface film but did not contribute materially to lump weight.

It is apparent that potatoes with a low moisture content or high dry matter content had a firmer texture and a tendency to form higher amounts of hard tissue. It is well known that tubers with high solids or dry matter content have high specific gravity and high starch content, and give a mealy texture after cooking (Chung and Hadziyev, 1980). Starch is a major constituent of potato and has significant effect on potato texture. Barmore (1937) found that tubers with a high starch content were significantly firmer that those with low starch content. They also observed that areas of the tuber having a low starch content became softer on cooking than areas with a high starch content. Mashed potatoes prepared from tubers with high and low specific gravity exhibited differences in texture. The number of bound cells and broken cells were greater in the low specific gravity samples than in the high specific gravity samples, which could result in different texture and appearance (Kaishi, 1991). Presumably, formation of lump tissues would be different between high and low density potatoes.

Starch probably has an effect on hard tissue formation during storage. Potatoes with high starch or solid content have a greater tendency to form hard tissue after prolonged storage than those with lower a solid content. It is possible that other factors besides starch, such as cell size, amylose and amylopectin content or presence of organic acids such as phytic acid, may also affect hard tissue formation. Unfortunately, these factors were not examined.

2.3.3. Effect of packaging conditions and storage time

Changes in color were detected in day 2 of storage for all samples as indicated by the change of a* and L* values as shown in Table 2.3. Visual observations of the development of discoloration during storage were generally consistent within reflectance data of L* and a*. Potatoes strips in all samples became darker as indicated by the decrease in L* value. Browning of the strips gradually increased during storage as indicated by the increase in a* value. Samples packaged in air and modified atmosphere (MAP) with 20% CO_2 + 5 % O_2 became unacceptable due to the discoloration only after 2 d, with similar changes in a* and L* values. However, no unacceptable color changes was found on samples packaged in vacuum and MAP with 20% CO_2 + 80% N_2 over 2 weeks. Figures 2.2 and 2.3 illustrate the changes in L* and a* values for all treated samples packaged in different conditions and stored at 4°C. No off-flavor was detected in all samples after 2 week of storage.

Exposure of potatoes to high oxygen level resulted in higher degree of browning. It was quite clear that treating pre-peeled potato strips with 2% CA and 1% AA and 500 ppm EDTA solution alone could not prevent discoloration. Only a combination of this treatment and MAP at very low O_2 atmosphere (<5%) or vacuum packaging is effective against discoloration. Having almost no oxygen initially inside the packages is advantageous as it can slow browning rate while O_2 level is decreasing during storage in air package. Similar results were found by Gunes and Lee (1997) and O'Beirne and Ballantyne (1987). When vacuum packaging was used without antibrowning inhibitor treatment, potato strips were found to retain excellent color for at least 14 days (O'Beirne and Ballantyne, 1987).

Table 2.3. Color of raw potatoes stored at 4 °C after mashing: effects of packaging atmosphere conditions and storage time

Storage time	Air Packaged			MAP(20%CO$_2$/5% O$_2$)			MAP(20% CO$_2$/80%N$_2$)			Vacuum Packaged		
	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*
Day 0	74.61	-2.31	16.46	74.61	-2.31	16.46	74.61	-2.31	16.46	74.61	-2.31	16.46
Day 2	70.69	-0.33	15.28	71.64	-0.57	15.98	73.09	-0.55	14.75	71.86	-1.41	15.42
Day 4	71.36	-0.19	16.33	70.54	-0.03	15.78	74.26	-0.8	15.34	74.13	-1.11	16.3
Day 6	69.55	0.16	16.95	70.8	0.49	15.74	70.19	-0.93	16.57	71.69	-1.05	15.69
Day 8	69.46	0.02	17.17	68.13	0.16	16.17	70.71	-1.3	15.76	71.13	-1.27	16.17
Day 10	68.01	-0.03	16.99	68.5	0.37	15.76	71.59	-1.16	16.42	70.36	-0.99	15.4
Day 12	68.74	-0.02	15.21	69.37	-0.06	15.02	71	-1.16	16.4	74.88	-1.23	15.31
Day 14	68	-0.33	15.16	66.11	0.89	14.99	70.36	-1.2	16.91	73	-0.87	15.57

Figure 2.2. L* values of raw potatoes stored at 4 °C after mashing: effects of packaging atmosphere conditions and storage time

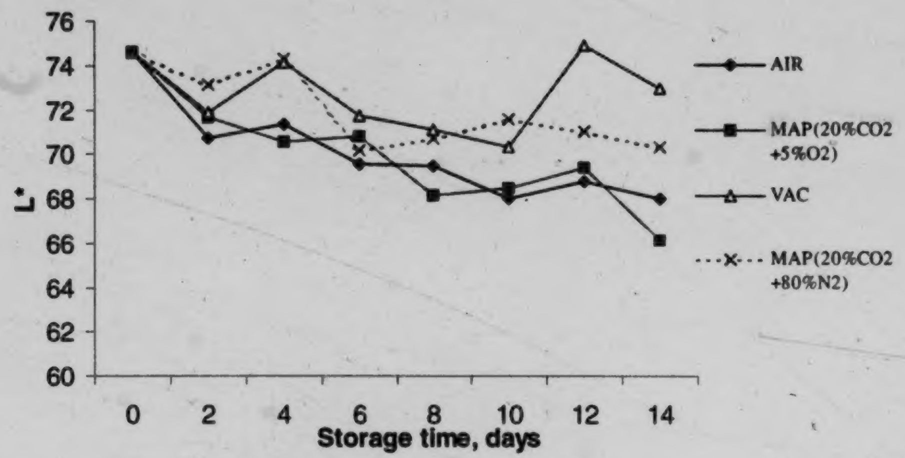
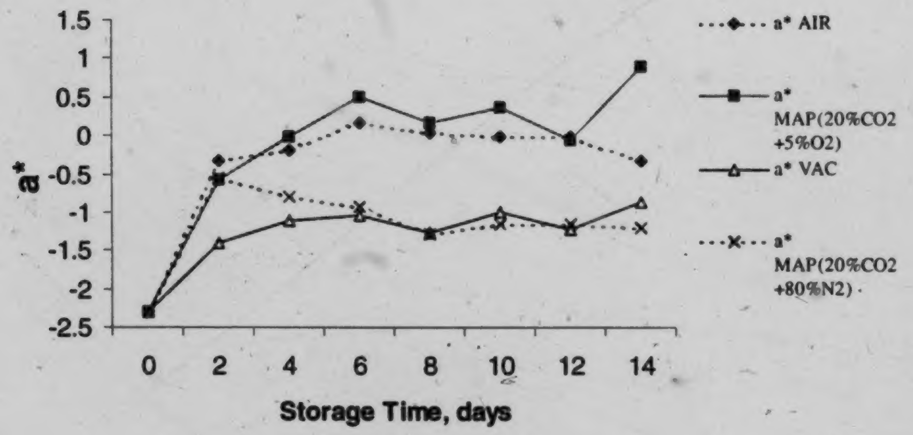


Figure 2.3. a* values of raw potatoes stored at 4 °C after mashing: effects of packaging atmosphere conditions and storage time



Changes in internal O_2 and CO_2 concentrations for all treated samples packaged in different conditions and stored at 4°C are shown in Figures 2.4 and 2.5. Due to an active modification of the atmosphere inside packages, most of the samples had a similar level of O_2 and CO_2 with the exception of air packaged samples. Reduction of O_2 inside the packages occurred in all samples. Oxygen levels in air packaged samples decreased rapidly during the first two days of storage. Processing and packaging cause changes in plant metabolism resulting in a noticeable increase of respiration rate (Gunes and Lee, 1997), thus the rapid reduction of O_2. Rapid changes in O_2 level were not found in samples packaged in low O_2 or without O_2. This is probably due to suppression of plant metabolism under these conditions. The oxygen level was stable after four days of storage. For samples packaged in MAP with 5% O_2 , oxygen slightly decreased until it was stable at around 2%, similar to the air packaged samples. For samples packaged in MAP without O_2 , O_2 level slightly increased during the first two days, then decreased, and became stable at the level a little lower than the other two samples. The initial increase was mostly likely due to O_2 from outside permeating into the package during the first two days of storage. It was quickly used for respiration, and because the plastic bag used had low O_2 permeability, an equilibrium was subsequently established between the permeation rate and the respiration rate, resulting in a low but stable O_2 level in the package.

CO_2 level in air packaged samples increased steadily throughout the 14 d of storage. While CO_2 levels increased slightly for MAP samples throughout the 2 wk storage. CO_2 level for samples in MAP with 20% CO_2 + 5% O_2 was slightly higher than those in MAP with 20% CO_2 + 80% N_2. The higher CO_2 level must be due to the slightly

Figure 2.4. Changes in CO_2 concentration in the packages of fresh potato strips dipped in antibrowning solution and packaged in various atmospheres

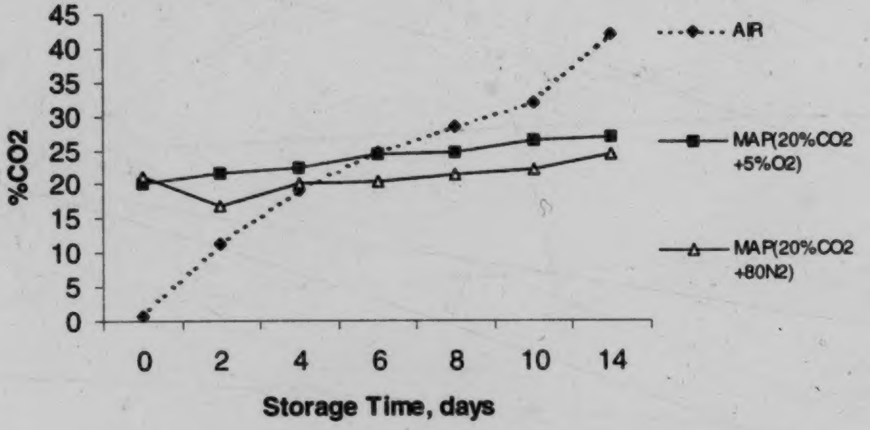
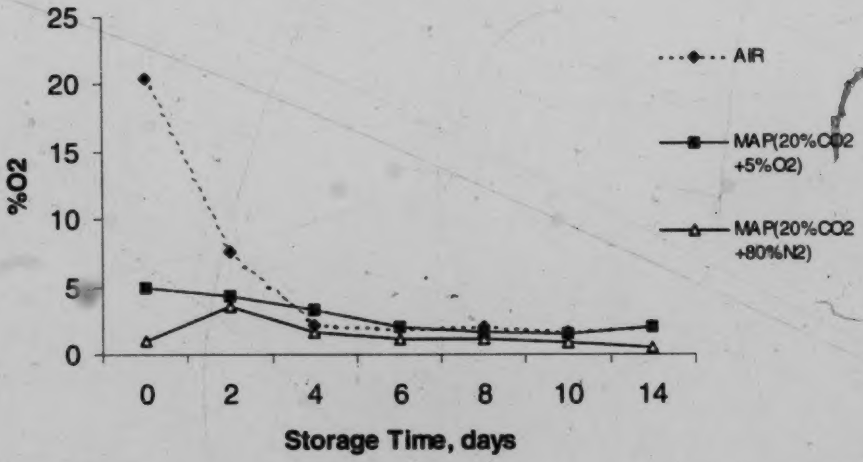


Figure 2.5. Changes in O_2 concentration in the packages of fresh potato strips dipped in antibrowning solution and packaged in various atmospheres



higher respiration rate under the initial 5% O_2 (which subsequently stabilized at lower level).

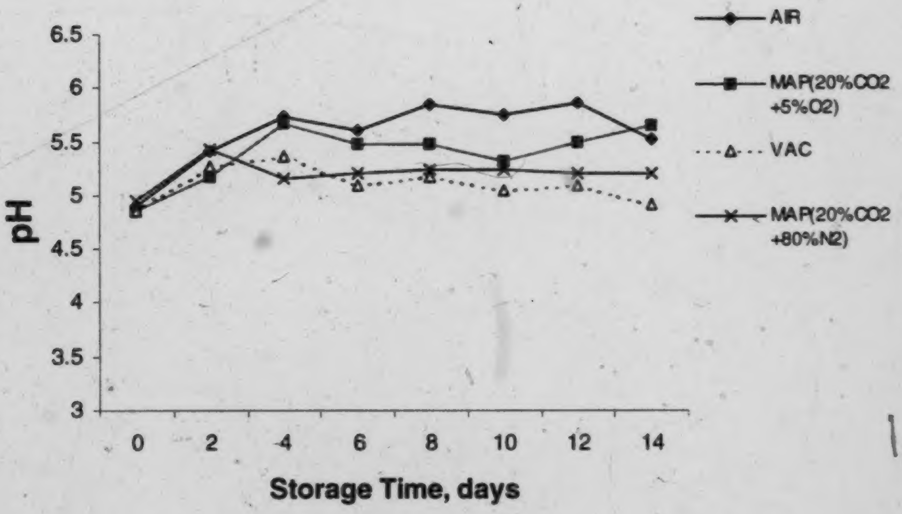
In a study of respiration rate of potato strips, Gunes and Lee (1997) found that decreasing O_2 level from 21% to 3% resulted in approximately 4-fold reduction in respiration rate. This was suggested to be due to the reduction in the activity of oxidases such as polyphenol oxidase (PPO), ascorbic acid oxidase and glycolic acid oxidase (Kader, 1986). Elevated CO_2 also reduces respiration rate, and CO_2 was reported to inhibit some steps in the Krebs cycle through the inactivation of some enzymes (Kader, 1986).

Figure 2.6 shows the pH of samples under various packaging conditions over 14 d storage. There was a slight increase in pH during the first four days of storage in samples packaged in air and in MAP with 20% CO_2 + 5% O_2. The pH of both samples remained relatively stable after day 4, with that of MAP samples slightly lower than that of air packaged samples. For samples packages in vacuum and MAP with 20%CO_2 + 80% N_2, there was little change in the pH over the 14 d storage.

One of the important effects of CO_2 is to increase acidity in plant tissues. Because intracellular pH values are normally regulated within narrow limits, only elevated CO_2 concentrations (as high as 5%) will lower intracellular pH (Salunkhe *et al.*, 1991). In MAP samples, CO_2 could dissolve into the potato tissue, resulting in a lower pH than that of samples packaged in air.

A lower pH of potato strip packaged under vacuum could be expected because high vacuum inside the packages would enhance the diffusion of dipping solution into the tissues. It has been reported that application of browning inhibitors to peeled potatoes

Figure 2.6. Change of pH of potato tissue during storage of potato strips under various packaging conditions

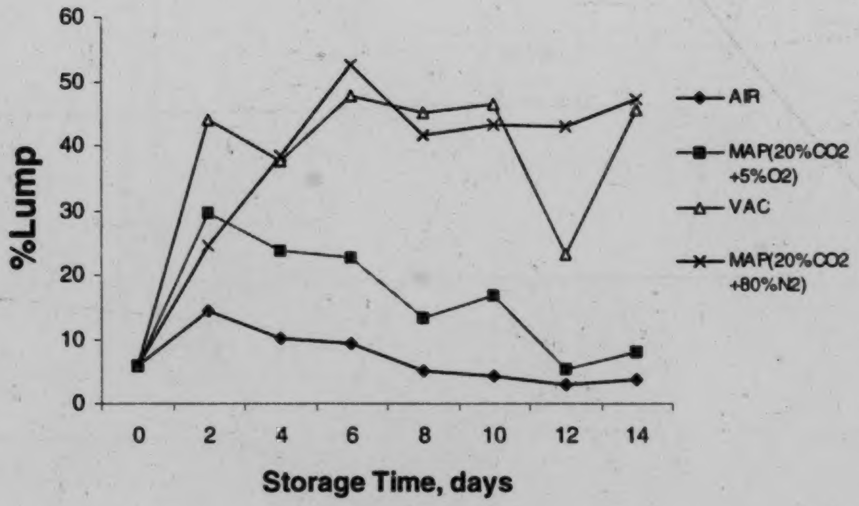


could be more effective by vacuum and pressure infiltration (Saper *et al.*, 1990).

It has been found that beansprouts packaged in lower permeability films had lower pH than those packaged in films of higher permeability. This acidic pH was correlated to the accumulation of lactate as a result of the prevailing action of heterofermentative lactic acid bacteria (Varoquaux *et al.*, 1996). However, in general, the number of microorganisms in sliced potatoes packaged in MAP and air is less than that of beansprouts and the growth rate is slower; therefore the number of microorganisms did not increase very much during storage (Gunes *et al.*, 1997; Ahvenainen *et al.*, 1998; Laurila *et al.*, 1998). It has also been reported that microbial populations such as aerobic bacteria, lactic acid bacteria or coliforms in potatoes packaged in MAP with 100% N_2, and in air did not differ significantly during storage (Gunes *et al.*, 1997). Therefore, the lower pH of samples packaged in MAP or vacuum was more likely to be caused by the diffusion of CO_2 or antibrowning solution into the potato tissue rather than by acid accumulation from bacterial activity.

Amounts of hard lump developed in potato samples treated with the same antibrowning solution and packaged in different atmospheric conditions are illustrated in Figure 2.7. The highest lump percentage was found in vacuum packaged samples, and MAP with 20% CO_2 + 80% N_2. MAP with 20% CO_2 + 5%O_2 gave lower amount of lump than the first two samples, while air packaged samples developed the least amount of lumps. In fact, lump formation in air packaged samples and in MAP with 20% CO_2 + 5%O_2 decreased considerably over the 14 d storage. A similar trend was not found in vacuum-packaged, and MAP samples with 20% CO_2 + 80% N_2 in which the amounts

Figure 2.7. Development of hard tissue during storage of potato strips under various packaging conditions



of hard tissue remained quite stable after they had been formed. It appeared that hard tissue formation took place during the first two days of storage in all samples. During storage, the degradation of insoluble protopectins to the more soluble pectic acid and pectin has been suggested in some fruits and vegetables and this can affect texture and appearance of the produce (Salunkhe *et al.*, 1991). Pectin degradation could occur during storage in air and MAP with 20% CO_2 + 5%O_2. This could explain the decrease in hard tissue formation in these samples. In samples packaged in MAP without O_2 or in vacuum, no or little pectin degradation occurred since pectin degradation could be retarded under this condition.

Hard tissue formation was more severe when vacuum and MAP were used, although these conditions were advantageous in preventing discoloration. Low tissue pH found in these samples could play a role in hard tissue formation. Many studies have found that low pH can increase firmness of plant tissue by inhibiting pectin degradation from β-elimination mechanism during cooking process (Albersheim *et al.*, 1960; Doesburg, 1961; Sterling, 1968; Fuchigami, 1983a; Fuchigami, 1983b; Sasaki *et al.*, 1999). Therefore, in samples with low pH pectin degradation during cooking could be slower resulting in more lump tissues after mashing.

Low oxygen reduces respiration rate with unknown metabolism (Kader *et al.*, 1989). The decline in respiration rate may be a response to a metabolic depression, which diminishes the demand for biological energy and in turn delays senescence (Solonos, 1994). High CO_2 and low O_2 could also cause numerous biological changes in plant tissues. Subjecting living plant tissue to this condition will result in stress, which is manifested as various symptoms, such as increasing respiration rate or ethylene production, or other physical disorder (Salunkhe *et al.*, 1991; Varoquaux and Wiley, 1994), and may result in the formation of hard tissues.

An important effect of CO_2 is the increased acidity in plant tissues. Intracellular pH values are normally regulated within narrow limits, therefore, elevated CO_2 concentrations will lower the intracellular pH (Salunkhe *et al.*, 1991). CO_2 is a metabolically active molecule participating in a number of carboxylation reactions, and this may affect plant metabolism. For example, dissociation of carbonic acid into bicarbonate and hydrogen ions could affect the activity of enzymes by inhibiting some of them, e.g. succinodehydrogenase or cytochrome oxidase (Varoquaux and Wiley, 1994). Dissociation of CO_2 is much greater when stored at low positive temperature than at ambient temperature (Salunkhe *et al.*, 1991). The acidification could result in either the reduction of enzyme activity or phytotoxicity of CO_2. Some enzymes regulating the texture of potatoes might be affected under this condition, resulting in adverse effects such as firmer or hard to cook tissues.

It has been suggested that modifications in conventional atmosphere may result in an alteration in metabolism, inducing some physiological changes (Do and Salunkhe, 1975). Kader (1986) found that high CO_2 concentrations reduced texture loss in strawberries. This was due to the improvement of physiological conditions compared to normal air storage. Physiological effects of high CO_2 concentration may vary. One effect is the reducing in the breakdown of pectic substances (Salunkhe *et al.*, 1991), which was speculated to be one cause of hard tissue formation.

2.4. Conclusion

Various antibrowning agents had different effects on hard tissue formation. However, since untreated samples also formed some hard tissues, other factors, besides antibrowning treatment, could also play a significant role in this problem. It is possible, therefore, that hard tissues develop during low temperature storage whether antibrowning treatment was applied or not.

Potatoes with higher solid content (or lower moisture content) developed a higher amount of hard tissue than those with lower solid content.

Pre-peeled potato strips packaged in vacuum or modified atmosphere with low O_2 concentration and high CO_2 concentration and stored at refrigerated temperature formed a higher amount of hard tissue than those packaged in air.

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CHAPTER 3. PHYSICOCHEMICAL CHANGES IN POTATO STRIPS PACKAGED UNDER VARIOUS ATMOSPHERIC CONDITIONS

3.1. Introduction

Two major components influencing potato texture are the cell wall and starch. Therefore, changes in these components could affect textural properties. Pectic substances make up about 52-55% of the potato cell wall/middle lamellae (Hoff and Castro, 1969; Jarvis *et al.*, 1981). The high proportion of pectic substances in the cell wall and middle lamellae emphasizes their importance in potato texture. Moreover, it has been known that ions affect texture by their interaction with pectic substances and increase cell wall strength. Besides the cell wall, starch also plays a major role in potato texture when they are cooked. The objective of this experiment was to determine the possible causes of hard tissue formation during storage. In this experiment, changes in cell wall components were investigated by comparing the amounts of soluble- and insoluble pectins and divalent ions in the cell wall of raw potato strips packaged in air and under vacuum and stored at refrigerated temperature. Changes in starch properties of fresh and stored samples were also investigated using a differential scanning calorimeter (DSC).

3.2. Materials and methods

Three different types of potatoes were used in this experiment:

Russet potatoes (Morinville, AB), designated Group 1

Round white potatoes (Canada and US grown), designated Group 2

Table potatoes (Canada and US grown), designated Group 3

They were purchased from local stores and held at 4°C until used.

3.2.1. Changes in moisture content, pH and lump percentage

Prior to peeling, potatoes were washed with water and sanitized by dipping in 100 ppm sodium hypochlorite solution for 1 min, and rinsed briefly with water. Washed potatoes were peeled by hand with a potato peeler. Following the peeling, potatoes were briefly submerged in cold water, cut into French-fried shapes with a cross-section of 1x1 cm by a Hobart slicer and submerged in distilled water until browning inhibitor treatment could be applied.

Strips of many tubers were mixed together before being divided into 600 g samples and treated with the solution of 1% ascorbic acid, 2% citric acid and 500 ppm EDTA. After draining, samples were either air-packaged in 2 mil LDPE bag or vacuum-packaged in a vacuum-type plastic bags, with Multivac type AG500 (Sepp Haggenmuller KG., Germany) packaging equipment. Strips without antibrowning treatments (control) were prepared by dipping in distilled water for 10 min and packaged in air. The samples were stored in a cooler at 4°C until determinations were performed.

At day 0 and day 7 of storage, all samples were analyzed for moisture content, pH and lump percentage using the procedures described in section 2.2.2.2., 2.2.1.1. and 2.2.1.2., respectively.

3.2.2. Changes in firmness

For each type of potato, two tubers of the same size were chosen. Each tuber was washed, peeled, and cut into strips. Only strips from the inner areas were used. Each strip was cut into cubes of 1x1x1 cm. All cube samples were mixed and divided into five groups. The first group was measured for firmness at once (fresh, day 0). The second group was packed in 2 mil LDPE bags and kept at 4°C. The third group was dipped in enzyme inhibitor solution and packaged in air in 2 mil LDPE plastic bags and stored at 4°C. The fourth group was prepared the same way as the third samples, but packaged under vacuum in vacuum bags and stored at 4°C. The last group was treated with enzyme inhibitor solution and was measured for firmness at once (treated, day 0). Firmness test was done after 7 days of storage for the second, third and forth groups.

3.2.2.1. Determination of firmness of raw potato tissue

Tissue firmness was measured with Instron Texture testing equipment (model 4201, MA, USA). The sample (1x1x1 cm) was placed on the load-cell and the plunger was driven downward onto the sample until it was compressed to 2/3 of its original height. The load cell used in this test was 50 kg. The plunger was immediately reversed upward to the original position above the sample surface. The maximum force applied to the sample was recorded as the firmness of the sample.

3.2.3. Changes in pectic substances

For each type of potato, two tubers of the same size were chosen. Each tuber was washed, peeled, then cut into strips. Only strips from the inner areas were used. All strip samples were mixed and divided into four groups. The first group was measured for pectic substance content at once (day 0). The second group was air-packaged in 2 mil LDPE bags without enzyme inhibitor treatment. The third group was dipped in enzyme inhibitor solution and packaged in air in 2 mil LDPE bags. The fourth group was prepared as in the third group but packaged under vacuum. The last three groups were stored at 4°C for 7 d when they were again analyzed for pectic substances.

3.2.3.1. Pectic substance determination

a). Preparation of samples for extraction

The method of Walter Jr. *et al.* (1992) was followed. The alcohol-insoluble solids (AIS) were prepared by blending 25 g sample of potato strips with 85 mL of 95% methanol and 11 mL of water. The mixture was then centrifuged (Model S2K, International Equipment Company, Mass., USA), and the supernatant was removed. The residue was extracted twice by boiling in 150 mL of 80% ethanol. The extracted residue (AIS) was freeze-dried and held in a dessicator until used.

b). Extraction procedure for water- and oxalate-soluble fractions.

For water-soluble fraction, the 0.2 g sample of AIS was shaken with 10 mL of distilled water for 10 min at 300 rpm in a shaker (New Bruswick Scientific Co., Inc., NJ, USA), following the method of Walter Jr. *et al.* (1992). The mixture was centrifuged, and the supernatant collected. The residue was extracted twice more in the same way. The second and the third supernatants were added to the first and diluted to 50 mL.

For oxalate-soluble fractions, the method of Walter Jr. *et al.* (1992) and Luh (1954) were modified. The residue from the water extraction was extracted with 10 mL of 0.2% ammonium oxalate (analytical grade, Sigma Chemical Co., MO, USA). The mixture was centrifuged, and the supernatant was collected. This procedure was repeated four times. All supernatants were mixed and diluted to 50 mL.

c). Extraction procedure for HCl-soluble fraction.

The residue from water and oxalate extraction was further extracted with 0.05N HCl (analytical grade, Fisher Scientific, NJ, USA) at 85°C for 2 h using a modified method of Bettelheim and Sterling (1955). The mixture was centrifuged, and the supernatant collected and dilute to 50 mL.

d). Extraction procedure for total pectin

The method of Hou and Chang (1996) was employed. A 0.05-g portion of AIS was extracted with 25 mL of 0.05 N HCl in a boiling water bath for 4 h. The mixture was centrifuged, and the supernatant collected and dilute to 50 mL.

e). m-Hydroxydiphenyl reaction method for the analysis of galacturonic acid content of potatoes

The method of Kintner *et al.* (1982) was employed. Galacturonic acid standard solutions were prepared from a stock solution of 1 mg/mL galacturonic acid (Sigma Chemical Co., MO, USA). A stock solution was made up with distilled water and kept refrigerated at 4°C. A new solution was made each month. Standard solutions containing 10, 20 and 30 µg/mL galacturonic acid were prepared as needed. 0.5 mL portions of standards and samples were pipetted into 16x150 mm test tubes and placed in an ice water bath to cool. After allowing to cool for several min, 3 mL of sulfuric acid

/tetraborate solution, prepared by making a solution of 0.0125 M sodium tetraborate (analytical grade, Acros Organics, NJ, USA) in concentrated sulfuric acid (analytical grade, Fisher Scientific, NJ, USA), was added to each of the tubes in the ice water and mixed carefully using a Vortex mixer (model k-550-G, Scientific Industries Inc., NY, USA) at moderate speed. The vortex mixer was started and stopped several times in order to mix samples well. The tubes were heated in a 100°C water bath for precisely 5 min and immediately placed in the ice-water to cool. A 0.05-mL aliquot of 0.15% m-hydroxydiphenyl (analytical grade, Acros Organics, NJ, USA) was added to develop color. After the m-hydroxydiphenyl addition, individual tubes were vortexed thoroughly. Tubes were allowed to sit 20 min at room temperature to allow the bubbles to dissipate. A red chromagen was formed in the presence of uronic acid. Since carbohydrates also produce a reddish chromagen when heated in sulfuric acid/tetraborate, a sample blank was made by replacing the m-hydroxydiphenyl reagent with 0.05 mL of 0.5% sodium hydroxide (analytical grade, Acros Organics, NJ, USA), keeping the rest of the procedure the same. Interference of carbohydrates was normally present in the HCl extracted fraction. The ratio value of 1.35 was used to multiply the absorbance of the sample blank to give a corrected sample blank absorbance according to Kintner and Van Buran (1982) and Van Marle *et al.* (1994). The corrected sample blank absorbance was later substracted from the total absorbance to obtain the absorbance due to m-hydroxydiphenyl. Absorbance reading values were used to find galacturonic concentration from a standard curve. Absorption measurements were taken at 520 nm using a Spectrophotometer (Spectronic 21, Bausch & Lomb). A reagent blank containing 0.5 mL distilled water, 3 mL sulfuric acid/tetraborate solution and 0.1 mL of 0.05%

sodium hydroxide was used to calibrate all instruments. Galacturonic acid concentrations were expressed as g/1000 g of dry matter and designated total pectin.

3.2.4. Changes in calcium and magnesium content

For this experiment, all samples were prepared in the same way as described in section 3.2.3. Samples of air-packaged and vacuum-packaged were stored at 4°C for 7 d. Calcium and magnesium contents in both the cell wall and starch were determined for both stored and freshly prepared samples.

3.2.4.1. Cell wall separation

Cell wall samples were prepared according to modified processes of Bartolome and Hoff (1972) and Liu *et al*. (1992). Mashed fresh potato samples and lump samples of 30 g each were homoginized with 100 mL of 0.1% cysteine (Sigma Chemical Co., MO, USA) solution with a high speed blender (Waring model 31BL92, Connecticut, USA) for 10 min at high-speed. The cell wall was collected by filtering the homogenized sample through two stainless standard screens with mesh size No. 70 and No. 170 (The W.S. Tyler Co. of Canada, Ltd., ON). The cell wall material was collected from the No. 170 screen and washed several times with deionized water until most of the starch was removed, as determined by a light microscope (Walter A. Carveth Ltd., Vancouver, B.C). To further remove residual starch, the cell wall material was transferred to a beaker and sonicated for 10 min, while maintaining the temperature at 25°C by adding ice, with Sonic 300 Dismembrator (Artex Systems Corp., NY, USA). The material was then transferred back to the No. 170 screen and washed several times with water and then freeze-dried. The first four filtrates through No. 170 screen were collected. Starch particles were separated with centrifugation and freeze dried.

3.2.4.2. Calcium and magnesium content determination

Determination of Ca^{2+} and Mg^{2+} in the freeze-dried cell wall and starch was carried out by atomic absorption following Alonso *et al.*, 1997. Dry samples (5-10 mg) were ashed at 500°C for 17 h, dissolved in 0.5 mL HNO_3 (ACS, BDH Inc., ON) and 0.5 mL 5%$LaCl_3$ (analytical grade, Sigma Chemical Co., MO, USA), brought to 25 mL with 1N HNO_3 and analyzed for Ca^{2+} and Mg^{2+} using atomic absorption spectrophotometer (model 4000, Perkin-Elmer, CT, USA). An air-acetylene flame was used for detection of both elements, measuring at 422.7 nm for Ca^{2+} and 285.2 nm for Mg^{2+}. Absorbance reading values were used to find Ca^{2+} and Mg^{2+} concentrations from a standard curve prepared from Ca^{2+} reference solution 1000 ppm±1% and Mg^{2+} reference solution 1000 ppm±1% (analytical grade, Fisher Scientific, NJ, USA).

3.2.5. Thermal properties of starch isolated from potato samples

All samples were prepared in the same way as in Section 3.2.3. After potato samples were stored at 4°C for 7 d, starch was isolated from strips and the thermal properties of isolated starch were performed by differential scanning calorimeter (DSC). The values from these stored samples were compared with those of freshly prepared potato strips.

3.2.5.1. Isolation of starch

Potato strips were pulverized in a Waring blender with distilled water for 2 min at high speed. The slurry was then homogenized for 5 min (Homogenizer, Kinematica GmbH, Switzerland). The mixture was centrifuged and supernatant removed. Twenty-five mL of 0.05N NaOH (analytical grade, Acros Organics, NJ, USA) was added to isolated starch. The mixture was shaken well for 5 min and centrifuged to remove NaOH solution. Isolated starch was washed 3 times with distilled water and centrifuged to isolate starch from water. The starch was vacuum dried at 50°C for 5 h (Vacuum dryer oven, National Appliance Co., OR, USA). Dry samples were ground and sifted though a standard sieve (60 mesh) in order to get a uniform particle size of starch powder.

3.2.5.2. Differential scanning calorimeter

Transition temperatures of isolated starch samples were measured and recorded on a differential scanning calorimeter (DSC) (model 910, Du Pont De Memours & Co., DE. USA). Water (21 μL) was added with a microsyringe to starch (6-7 mg dry basis) in the DSC pan (Wested Industrial Ltd., ON) then sealed and kept at room temperature for more than 1 h. The scanning temperature range and the heating rate were 30-100°C and 5°C/min, respectively. An empty pan was used for calibration. The transition temperatures recorded were the onset (To), peak (Tp) and conclusion (Tc) temperatures of the gelatinization endotherm. The enthalpy of gelatinization (ΔH) was estimated by integrating the area between the thermogram and the base line under the peak, and was expressed in terms of joules per unit weight of dry starch (J/g). All DSC experiments done were replicate determinations.

3.3. Results and Discussion

3.3.1. Changes in moisture content, pH and lump formation

The moisture content of the three groups of potatoes, packaged in air and in vacuum, at days 0 and 7 are shown in Table 3.1. The Russet potatoes (Group 1) had a moisture content of 76%, 81% was the contents of the round white potatoes (Group 2) and 83% for the table potatoes (Group 3). Therefore, the dry matter contents of the three types of potatoes were 24%, 19% and 17%, respectively. No significant change in moisture after 7 d was detected in Group 1, but Groups 2 and 3 appeared to gain moisture slightly.

During storage, water loss is expected. Loss of water is generally high after processing steps. Cutting and peeling a fruit and vegetable exposes interior tissues and dramatically increases the water evaporation rate (Brecht, 1995). Therefore, loss of water would be expected during prolonged storage. However, in the present experiment, packaging in plastic films appeared to prevent moisture loss in all samples very effectively.

Freshly prepared strips had similar pH values, in the range of 5.7-5.8. After dipping in antibrowning solution for 10 min, the pH dropped 0.5, 0.3, and 0.3 unit for Group 1, 2 and 3, respectively (Table 3.2). For all type of potatoes, pH of air-packaged samples increased ($p<0.05$) during storage, but that of vacuum-packaged samples decreased slightly. These results agreed with those found in previous experiments (Section 2.3.3) where lower pH was found on strips packaged under vacuum.

Table 3.1. Changes in moisture content of raw potato strips before and after storage in various packaging conditions for 7 d at 4°C

Potato	Fresh (Untreated)	Untreated, Air- packaged (day7)	Treated, Air-packaged (day7)	Treated, Vacuum-packaged (day 7)
Group 1	76.48 ± 0.33^{ns}	76.95 ± 0.06^{ns}	76.48 ± 0.34^{ns}	76.74 ± 0.11^{ns}
Group 2	$80.76\pm+0.4^{a}$	82.32 ± 0.94^{b}	81.66 ± 0.59^{b}	81.09 ± 0.53^{b}
Group 3	83.39 ± 0.21^{a}	84.48 ± 0.47^{b}	84.82 ± 0.14^{b}	84.58 ± 0.58^{b}

a,b Means not sharing the same superscript are different ($p<0.05$) for each group of potatoes

Table 3.2. Changes in pH of raw potato strips before and after storage in various packaging conditions for 7 d at 4°C

Potato	day0		day7		
	Untreated	Treated	Untreated, Air-packaged	Treated, Air-packaged	Treated, Vac-packaged
Group1 (76%moisture)	5.85 ± 0.04^{a}	5.3 ± 0.05^{b}	6.2 ± 0.02^{c}	6.1 ± 0.04^{c}	5.37 ± 0.03^{b}
Group 2 (81%moisture)	5.75 ± 0.01^{a}	5.4 ± 0.02^{b}	5.9 ± 0.05^{c}	5.65 ± 0.01^{a}	5.21 ± 0.04^{d}
Group 3 (83%moisture)	5.87 ± 0.02^{a}	5.5 ± 0.04^{b}	6.1 ± 0.03^{c}	6.16 ± 0.03^{c}	5.38 ± 0.02^{d}

a,b,c,d Means not sharing the same superscript are different ($p<0.05$) for each group of potatoes

Table 3.3. Hard tissue development in raw potato strips before and after storage in various packaging conditions for 7 d at 4°C

Potato	Lump Percentage			
	Fresh (Untreated)	Untreated, Air-packaged	Treated, Air-packaged	Treated, Vac-packaged
Group1 (76%moisture)	5.8 ± 1.32^{a}	11 ± 3.21^{a}	9.25 ± 3.94^{a}	47.75 ± 3.51^{b}
Group 2 (81%moisture)	4.8 ± 1.56^{a}	-	-	15.3 ± 2.21^{b}
Group 3 (83%moisture)	4.5 ± 1.34^{a}	-	-	14.4 ± 2.01^{b}

- no lump was detected

a,b Means not sharing the same superscript show difference ($p<0.05$) between fresh and stored potatoes for each group

Table 3.3 shows the percentage of hard tissue that developed after potato strips, packaged in air or vacuum, were stored for 7 d at 4°C. In all groups, about 4.5-5.8 % lumps were observed when samples were cooked and mashed. In Group 1 (76% moisture), hard tissues developed in both treated (9.25%) and untreated (11%) samples packaged in air. Vacuum packaging caused the highest amount of hard tissue formation (about 47%) after 7 d. For Group 2 (81%moisture) and Group 3 (83%moisture), no lump was detected in both treated and untreated samples packaged in air, after 7 d. Most tissues from these samples collected on the screen were brown thin film developed on the surface. These tissues could not be mashed and did not pass through the screen, but they were not considered lump tissue. However, obvious lump tissues were observed when these potatoes were treated with antibrowning solution and packaged in vacuum; 15% and 14% for Group 2 and 3, respectively. During storage in normal atmosphere, the breakdown of insoluble pectin into soluble pectin is suspected during prolonged storage by polygalacturonase activity. However, this occurs at a slower rate or is retarded by vacuum packaging or MAP owing to the reduction of polygalacturonase activity. As a result, lump tissues were not detected in air-packaged samples but found in higher amounts in vacuum-packaged samples during storage. In vacuum packaged samples, lump percentage was found to be significantly higher in potatoes with a low moisture content. These results were similar to those in the previous experiment (Section 2.3.3) in that vacuum packaged potatoes with high solid contents developed more hard tissue.

3.3.2 Changes in firmness

Table 3.4 shows compressive resistance (firmness) values of fresh and stored raw potato strips, packaged in air or vacuum and stored 4°C for 7 d. Results of freshly

Table 3.4. Compressive resistance (kgf) of raw potato strips before and after storage in various packaging conditions for 7 d at 4°C

Potato	Day 0		Day 7		
	Untreated	Treated	Untreated, Air-packaged	Treated, Air-packaged	Treated, Vac-packaged
Group 1.	16.60 ± 5.56^{a}	18.83 ± 3.59^{a}	24.32 ± 1.05^{b}	18.32 ± 1.74^{a}	25.01 ± 2.33^{b}
Group 2.	12.3 ± 1.32^{a}	13.14 ± 0.62^{a}	20.77 ± 3.35^{b}	17.3 ± 3.77^{c}	23.16 ± 3.10^{b}
Group 3.	15.49 ± 2.44^{a}	15.72 ± 3.02^{a}	25.41 ± 1.91^{b}	21.75 ± 5.02^{a}	24.42 ± 0.30^{b}

a,b,c: Mean scores in the same row not sharing the same superscript are significantly different ($p<0.05$)

prepared raw strips showed that Group 1 potatoes had the firmest texture (16.6 kgf), Group 2 had the lowest value (12.3 kgf), while Group 3 was in between (15.49 kgf). These appeared to agree with McComber *et al.* (1988) who found that shear strength of cooked Russet Burbank was higher than that of Norchip, Pontiac and LaSoda. No significant changes in firmness was found on raw potato strips after treatment with antibrowning solution, giving the firmness values of 18.83, 13.14 and 15.72 kgf for Group 1, 2 and 3, respectively.

After 7 d, the firmness significantly increased ($p<0.05$) in every treatment. For all types of potatoes, the firmness of the untreated, air-packaged samples and the treated, vacuum-packaged samples increased the most, and the treated, air-packaged samples increased the least. The reduction of pH in the antibrowning-treated and air-packaged samples may explain its lower firmness. Breakdown of pectic substances has been shown possible at very low pH, conditions such as in the antibrowning solution with a pH about 2 (Albersheim *et al.*, 1960; Doesburg, 1961; Sterling, 1968; Fuchigami, 1983a; Fuchigami, 1983b; Sasaki *et al.*, 1999).

The results did not show a clear relationship between lump percentage and compressive strength, except for Group 1 potatoes. Group 1 potatoes packaged under vacuum did not show significant difference in compressive strength from that of Groups 2 and 3 potatoes packaged in vacuum. On the other hand, their lump percentages were quite markedly different; 47% for Group 1 and 15% and 14% for Groups 2 and 3, respectively. Similarly, the firmness of untreated air-packaged and treated vacuum packaged Group 1 potato strips was not different, while their lump percentage was quite different. For Groups 2 and 3 potatoes, lump tissues were not found on untreated, stored samples while their compressive strengths were as high as the vacuum packaged samples, which contained very high amount of lump tissues.

However, it is apparent that, for all types of potatoes, vacuum packaged samples, which contained the highest amount of lump tissues, had the highest compressive strength values as well.

Groups 2 and 3 potatoes contained higher amounts of pectin than Group 1 potatoes because high dry matter content generally reflects a high amount of starch and low pectic substance content (Linehan and Hughes, 1969a; Chung and Hadziyev, 1980). It is well known that pectin affects the texture of potatoes; therefore, high pectin content in Group 3 potatoes resulted in firm texture in the raw samples (Table 3.5). However, lump percentage in cooked tissue, may not correlate with the firmness of raw tissue because most of pectins degrade during the cooking process. This could explain why no hard tissue was detected in treated and untreated samples of Groups 2 and 3 potatoes packaged in air, although difference in raw tissue firmness was found.

Generally, tissues became firmer after storage, although a relationship between firmness value and lump formation was not found. Van Buren (1979) suggested that increased resistance to deformation may be caused by changes in turgor or in pectin type or its quantity. It is possible that prolonged storage at low temperature, and packaging condition can be significant factors causing firmer tissue and probably lump formation.

3.3.3. Changes in pectic substances

Differences in total pectin were found in the three types of potatoes. Potatoes with low moisture content (76%) contained 5 mg/g of total pectin, the lowest amounts of pectin, as indicated in Table 3.5. Potatoes with highest moisture content. (83%) contained

Table 3.5. Contents of total pectin and pectic substance fractions extracted from raw potatoes strips before and after storage in various packaging conditions for 7 d at 4°C

Potato samples		Package	Galacturonic content (mg/g dry basis)				
			Water-soluble	Ammonium oxalate-soluble	HCl-soluble	Sum*	Total pectin
Group 1 (76% moisture)	tuber 1	Fresh	2.11	1.24	2.17	5.52	5.01
		Untreated	0.85	1.16	2.36	4.37	4.5
		Air (treated)	1.13	0.67	2.3	4.1	4.32
		Vacuum (treated)	0.74	1.18	2.53	4.45	4.48
	tuber 2	Fresh	2.34	1.38	2.41	6.13	5.97
		Untreated	1.05	1.24	2.76	5.05	5.27
		Air (treated)	1.82	1.15	2.63	5.6	5.86
		Vacuum (treated)	0.98	1.19	2.97	5.14	5.96
Group 2 (81% moisture)	tuber 1	Fresh	2.85	0.31	7.63	10.79	12.1
		Untreated	3.35	0.31	6.71	10.37	10.68
		Air (treated)	2.01	0.27	7.72	10	12
		Vacuum (treated)	1.66	0.27	8.23	10.1	12.81
	tuber 2	Fresh	3.18	0.34	7.95	11.47	14.21
		Untreated	2.82	0.39	6.9	10.11	10.36
		Air (treated)	2.28	0.29	7.93	10.5	13.66
		Vacuum (treated)	1.39	0.26	8.95	10.6	12.9
Group 3 (83% moisture)	tuber 1	Fresh	3.53	0.75	7.05	11.33	26.9
		Untreated	3.71	0.67	5.74	10.12	28.94
		Air (treated)	4.17	0.67	4.99	9.83	27.94
		Vacuum (treated)	2.68	0.42	6.7	9.8	25.98
	tuber 2	Fresh	3.15	0.97	6.17	10.29	26.29
		Untreated	3.52	0.88	5.88	10.28	27.08
		Air (treated)	3.69	0.63	6.58	10.9	26.57
		Vacuum (treated)	3.06	0.46	6.01	10	27.1

* Sum is a combination of water soluble-, ammonium oxalate soluble- and HCl soluble-fractions of pectins.

the highest total pectin content (27 mg/g), while potatoes with 81% moisture had total pectins of 13 mg/g. However, when the three pectic substance fractions of Group 3 potatoes were combined, the result was quite low when compared to its total pectin content. This was because when the pectin content is high, not all pectin is extracted from the samples during successive extraction. Since longer extraction time (2 h vs 4 h) was used when total pectin extraction was performed, this could result in the higher amounts of extractable pectin than when successive pectin extraction was performed.

The pectin contents in each fraction were similar between the two tubers taken from the same type of potatoes. The water-soluble fraction accounted for 38, 27, and 31% of total pectin extracted from Groups 1, 2 and 3 potatoes, respectively. The water-soluble fraction is characterized by a relatively high methyl content. This fraction is easily degraded during cooking (Bettelheim and Sterling, 1955). Ammonium oxalate-soluble pectic substances are the low-methyl pectinates of the polyvalent cations, magnesium and calcium. This fraction is derived from middle lamella and is quite high in calcium content (Dietz and Rouse, 1953; Bettelheim and Sterling, 1955). In the present study, this fraction accounted for a minor portion of the pectic material, 22, 3, and 8% of total pectin extracted from Groups 1, 2 and 3 potatoes, respectively. The last faction is the HCl-soluble fraction. The hot acid extraction will remove the remainder of the more deeply located, insoluble pectic substances, including calcium and magnesium pectates not removed by the ammonium oxalate extraction, and the protopectin. Protopectin is the water-insoluble parent pectic substance, which yields pectinic acids upon acid hydrolysis. Hot acid will solubilize the calcium and other insoluble salts, break hydrogen bonds and primary valent bonds (Dietz and Rouse, 1953; Bettelheim and Sterling, 1955). Pectic substance in HCl-soluble fractions accounted for 40%, 70% and 61% of the total pectic substances extracted from Groups 1, 2 and 3 potatoes and it was a major fraction of pectic substances found in all types of potatoes. Similar results have been found by other researchers (Bettelheim and Sterling, 1955).

Changes in pectic fractions of potato strips packaged in different atmospheric conditions are illustrated in Table 3.5. In Group 3 potatoes, which contained high amount of pectins, a significant decrease ($p<0.05$) in water-soluble pectin and an increase in HCl-soluble pectin were found in treated samples packaged in vacuum. No significant changes were found in pectic materials in the three fractions of treated and untreated samples packaged in air and stored for 7 d. Similarly, in Group 2 potatoes, pectic materials in the water-soluble fraction of treated samples packaged in vacuum significantly decreased ($p<0.05$) after storage, while HCl-soluble pectin increased. A decrease of water-soluble pectin was found in treated sample packaged in air but not in untreated samples similarly packaged.

In Group 1 potatoes, or low pectin potatoes, a significant decrease ($p<0.05$) in water-soluble pectin was found in both treated and untreated samples after storage. The highest decrease ($p<0.05$) of water-soluble pectin was found in vacuum packaged samples, followed by untreated and treated samples packaged in air.

Changes in pectic materials may be caused by several factors. In plant tissues, change in pectic substances was observed after they were subjected to severe conditions causing tougher texture. Kasai *et al.* (1997) found that root vegetables such as Japanese radish and carrots were hardened by the application of a high pressure of several hundreds MPa. In that study, water-soluble and HCl-soluble pectins decreased after the treatment, while chelating agent-soluble pectin significantly increased. High pressure treatment destroyed the cell membranes causing metal ions transferring to cell wall. Moreover, lowering the degree of esterification of pectin also occurred during the treatment. In this case, metal ions (Ca^{2+} and Mg^{2+}) binding with pectins could cause the decrease of soluble pectins resulting in firmer tissue. However, the authors suggested that the interaction among tissue components was also related to the hardening.

In the present study, the ammonium oxalate-soluble fraction did not change significantly after storage and even decreased in some cases. The bridges formed between metal ions and pectins causing the hardening would, therefore, be unlikely. Also, in vacuum packaging, vacuum, not pressure, was applied and, thus, would not cause a decrease in the degree of esterification.

Fruits or vegetables stored under MAP or vacuum packaging may undergo changes in pectic substances. Ptocharski (1982) and Smith (1992) showed that there are changes in pectin composition in strawberries subjected to CO_2 in controlled atmosphere storage. An increase in the quantity of pectin in the ammonium oxalate-soluble fraction and a decrease in the water soluble fraction contributed to firmer texture. An increase of insoluble pectin and firmness was also found in ciku stored in vacuum and MA packaging (Mohamed *et al.*, 1996). During storage in normal atmosphere, water-soluble pectin increases because protopectins are converted to soluble pectin and used as a substrate for respiration. However, this occurs at a slower rate or is retarded by vacuum packaging or MAP owing to the reduction of polygalacturonase activity. In air package and in MAP with 5% O_2, increases in sugars and acids as a result of the breakdown of insoluble pectin is suspected during prolonged storage. As seen from the results in

Section 2.3.3, lump percentage decreased when samples were packaged in air or MAP with 5% O_2 but remaining largely unchanged in samples packaged in vacuum or MAP with 20% CO_2. This may indicate the reduction in polygalacturonase activity under MAP and vacuum packaging.

Low pH in vacuum packaged samples could also interfere with the degradation of pectins. Possibly, reduced pectin beta-degradation occurred in these samples during cooking, resulting in hard tissues. An increase in hardness was found in stored cowpea seeds due to a decrease in tissue pH from 6.2 to 5.6 caused by reduced pectin beta-degradation during cooking (Liu *et al.*, 1992).

In Group 1 potatoes, it was found that stored samples that formed higher hard tissue contained lower water-soluble pectin. The lowest level of water-soluble pectin was found in vacuum-packaged samples, which had the highest lump percentage. There was no detectable hard tissue in treated and untreated Group 3 potatoes stored in air, and no change in pectin fractions was found. This appears to indicate that pectic substances and hard tissue formation are highly related.

It is apparent that prolonged storage and packaging methods can cause the transformation of pectic materials from water-soluble into water-insoluble form. This was clearly shown when treated samples were packaged in vacuum, in which water-soluble pectin decreased causing the formation of high amount of lump tissues.

Pectin has been found to influence the texture of cooked potatoes (Keijbets, 1974; Van Buren, 1979; Van Buren, 1991; Kunzek *et al.*, 1999). Upon cooking, pectin solubilization, middle lamella breakdown, cell wall loosening and starch gelatinization occur which affect potato texture (Van Marle *et al.*, 1997). Swelling of cell walls has been observed to help softening the texture during cooking (Shomer, 1995; Shomer *et al.*, 1995). If insoluble pectin increased during storage, tougher cell wall or less swelling could occur during cooking, resulting in a firmer texture.

Sharma *et al.* (1959), by using an objective measurement of tuber firmness, found that tubers with cooked hard texture contained less water-soluble pectic substances. Preheated and then cooked potatoes were found to contain higher insoluble pectin content and were firmer than cooked potatoes without preheating (Sasaki *et al.*, 1999).

Sharmar *et al.* (1959) suggested that an association of water-soluble pectin in cell wall with other components such as cellulose or hemicellulose probably caused the lowering of extractable water-soluble pectin fraction and the increase of insoluble fraction after potatoes were stored at low temperature. In that study, hemicellulose increased during cool storage. A decrease in water-soluble pectin fraction and an increase in water-insoluble fraction during cool storage was also found in mango fruits (Ketsa *et al.*, 1999), sweet potatoes (Baumgardner and Scott, 1963; Kattan and Littrel, 1963) and golden delicious apples (Klein *et al.*, 1995), exhibiting a firmer texture after cool storage. Association of water-soluble pectin with other components such as hemicellulose or phenolic compounds has been suggested to render water-soluble pectic fraction to be insoluble resulting in harder texture after cooking.

Cell membrane degradation during cool storage has been shown to be possible during storage of pre-peeled potatoes at low temperature. Enhancement of electrolyte leakage by chilling (Lieberman *et al.*, 1958) is accounted for by changes in the properties of phospholipids in cellular membranes (Lyons, 1973; Yamaki and Uritani, 1972; Yamaki, 1973; 1974). Chilling results in firmer roots of sweet potatoes after processing as compared to non-chilled roots (Baumgardner and Scott, 1963; Kattan and Littrel, 1963). Garcia *et al.* (1998) found that in stored beans exhibiting the hard-to-cook problem, more phenolics were associated with the soluble-pectin fraction. It was suggested that more ferulic acid bound to soluble pectin could result in changes in cell adherence, and consequently lead to a texture defect by impairing cell separation in hard beans. The increase in the protopectin accompanied by a firmer texture was found when nectarines (Mollendorff *et al.*, 1993) and peaches (Ben-Arie and Lavee, 1971) were stored at cool temperature. They proposed that this phenomenon was caused by the leakage of fluids and solutes through the membrane (as a result of cold storage) and the binding of these solutes to high-molecular-mass pectins (water-soluble pectins) to form pectin gels in the intercellular spaces, giving an increased level of water-insoluble pectin. Development of an intercellular matrix with new carbohydrates and pectins and appearance of cell wall synthesis were also observed in cold storage of peaches as a result of chilling injury (Ben-Arie and Lavee, 1971). Moreover, higher levels of water-insoluble pectic materials and hemicellulose were observed in potato tubers stored at low temperature, which exhibited harder tissue compared with unstored tubers (Sharma, 1959). Therefore, modifications of pectin fractions caused by association of pectic materials with other components providing cell-cell interactions that are stable to heat is highly likely to cause tissue-hardening problem.

In general, therefore, factors that could influence the development of hard tissue during prolonged storage would include the types of potatoes and packaging conditions. Potatoes with low pectin or high starch content were predisposed to form hard tissue during storage. Storage under vacuum condition could induce hard tissue formation. Hard tissue formation after detected cooking can be caused by a reduction of extractable water-soluble pectic materials and an increase in extractable insoluble-pectic materials.

3.3.4. Change in calcium and magnesium contents

Table 3.6 and 3.7 shows changes in Ca^{2+} and Mg^{2+} contents after 7 d of storage under various packaging conditions for different potatoes. Ca^{2+} and Mg^{2+} contents vary with types of potatoes. Potatoes with high pectin content (Group 3) contained 5.38mg Ca^{2+}/100g sample and 5.91mg Mg^{2+}/100g sample, higher than those with lower amounts of pectins containing 4.99 mg Ca^{2+}/100g sample and 5.0mg Mg^{2+}/100g sample for Group 1 potatoes, and 2.28mg Ca^{2+}/100g sample and 4.87mg Mg^{2+}/100g sample Group 2 potatoes.

For all types of potatoes, larger concentrations of Ca^{2+} and Mg^{2+} were found in cell wall and middle lamella. The rest was associated with starch. There was no significant change in the Ca^{2+} and Mg^{2+} content of cell walls isolated from treated and untreated samples

after 7 d storage in both air and vacuum packages. Neither was there a significant change in Ca^{2+} and Mg^{2+} content in starch isolated from the stored samples.

It has been suspected that the structural changes associated with case hardening and lump formation were initiated by heating. When heating was used to increase effectiveness of antibrowning treatment, the temperature can be in the range that activate pectin methylesterase (PME), resulting in partial demethylation of pectin and the creation of new binding sites for Ca^{2+} or Mg^{2+} (Bartolome and Hoff, 1972; Sapers *et al.*, 1997).

Table 3.6. Calcium content from cell wall and starch isolated from raw potato strips before and after 7 d storage under various packaging conditions at 4°C.

Sample		Calcium Content (mg/100 g wet basis)							
		Cell Wall				Starch			
		Day 0	Day 7			Day 0	Day 7		
		Fresh	Untreated	Treated (Air)	Treated (Vacuum)	Fresh	Untreated	Treated (Air)	Treated (Vacuum)
Group 1 (76% moisture)	Tuber 1	3.55		3.04	3.07	1.18		1.00	1.2
	Tuber 2	5.43		4.49	4.46	1.71		1.63	1.91
	Average	4.49^{ns}		3.76^{ns}	3.76^{ns}	1.44^{ns}		1.32^{ns}	1.56^{ns}
Group 2 (81% moisture)	Tuber 1	2.26	1.99	1.73	1.92	0.51	0.30	0.36	0.48
	Tuber 2	2.31	2.35	2.07	2.3	0.34	0.13	0.28	0.29
	Average	2.28^{ns}	2.17^{ns}	1.9^{ns}	2.11^{ns}	0.42^{ns}	0.22^{ns}	0.32^{ns}	0.38^{ns}
Group 3 (83% moisture)	Tuber 1	5.53	6.47	6.79	4.28	0.90	0.64	0.67	0.49
	Tuber 2	5.22	7.55	5.09	4.47	0.75	0.57	0.63	0.64
	Average	5.38^{ab}	7.01^{a}	5.94^{ab}	4.38^{b}	0.83^{a}	0.61^{ab}	0.65^{ab}	0.56^{b}

ns : Mean scores in the same category are not significantly different ($p<0.05$)

a,b : Means scores of calcium content in cell wall and starch in the same category not sharing the same superscript are significantly different ($p<0.05$)

Table 3.7. **Magnesium content from cell wall and starch isolated from raw potato strips before and after 7 d storage under various packaging conditions at 4°C.**

Sample		Magnesium content (mg/100 g wet basis)							
		Cell Wall				Starch			
		Day 0	Day 7			Day 0	Day 7		
		Fresh	Untreated	Treated (Air)	Treated (Vacuum)	Fresh	Untreated	Treated (Air)	Treated (Vacuum)
Group 1 (76% moisture)	Tuber 1	4.56		4.78	4.59	1.32		1.45	1.60
	Tuber 2	5.45		5.49	5.45	1.38		1.24	1.48
	Average	5.00^{ns}		5.14^{ns}	5.02^{ns}	1.35^{ns}		1.34^{ns}	1.54^{ns}
Group 2 (81% moisture)	Tuber 1	4.11	4.69	3.97	4.13	1.50	1.51	1.28	1.66
	Tuber 2	5.63	6.48	5.44	6.15	1.21	1.16	1.30	1.38
	Average	4.87^{ns}	5.58^{ns}	4.70^{ns}	5.14^{ns}	1.36^{ns}	1.34^{ns}	1.29^{ns}	1.52^{ns}
Group 3 (83% moisture)	Tuber 1	6.38	7.91	9.09	6.07	1.18	1.19	1.22	1.18
	Tuber 2	5.43	7.69	6.46	4.67	1.2	1.27	1.30	1.24
	Average	5.91^{ns}	7.80^{ns}	7.78^{ns}	5.37^{ns}	1.19^{ns}	1.23^{ns}	1.26^{ns}	1.21^{ns}

ns : Mean scores in the same category are not significantly different ($p<0.05$)

Diffusion of acids into the affected tissue could mobilize and redistribute Ca^{2+} (or Mg^{2+}) from cell interiors to cell walls so that cross-linking could occur (Sapers *et al.*, 1997). If cross-linking causes hard tissue formation, one could expect an increase of Ca^{2+} and Mg^{2+} in cell wall of stored potatoes. However, from the results in the present study, there was no change in the mineral content during storage. Moreover, heat treatment was not applied during antibrowning treatment, which could activate PME. Therefore, it did not appear likely that cross-linking between cell wall materials by Ca^{2+} and Mg^{2+} would be the major cause of hard tissue formation.

Hard tissue or lump formation was observed in pre-peeled sliced potatoes after storage at low temperature even when heat was not involved in the processing (Svensson, 1971). This problem was also reported after cooking of cool-stored unpacked tubers (Sharma *et al.*, 1959).

It is possible that there is a relatively poor apparent relationship between intercellular adhesion and Ca^{2+} and Mg^{2+} content since it is likely that, in the tubers, a large proportion of these ions complex with the organic chelating agents which exist in the tuber (Linehan and Hughes, 1969b). Thus, only a relatively small, and probably variable, proportion of the ions might be available to form cross-linkages within the intercellular cement of the cooked tuber. Nevertheless, in real term, these linkages were extensive enough to still contribute materially to the hard tissue formation.

3.3.5. Thermal properties of starch isolated from potato samples

The gelatinization transition temperatures (To, Tp and Tc) and the gelatinization enthalpy (ΔH) of starch isolated from fresh and stored potato strips packaged in air and vacuum, as measured by differential scanning calorimeter (DSC), are presented in Table 3.8. Gelatinization temperatures of isolated starch from various potatoes were 61.37, 61.77 and 62.51°C for Group 1, 2 and 3 potatoes, respectively. The gelatinization transition temperatures (To, Tp and Tc) differed ($p<0.05$) among individual tubers. Different components, including granule size, could affect the gelatinization pattern of starch in the tubers.

Only a single endothermic transition has been observed in the present experiment due to a high portion of water during the heating process. A representative DSC curve for potato starch is shown in Fig. 3.1. Upon heating, if a large amount of water is present (more than 80%), extensive hydration and swelling of the amorphous regions facilitate melting of the starch crystallites. This occurs over a very narrow temperature range, and, therefore, results in a single endothermic transition (Biliaderis *et al.*, 1980). In more concentrated starch solutions, two endothermics occur in which the destabilizing effect of the amorphous region decreases and, because of the limited amounts of water present, only partial melting of crystallites occurs according to the previous mechanism (first endotherm). Subsequent redistribution of the water around the unmelted crystallites will assist their melting upon further heating at a higher temperature (second endotherm) (Biliaderis *et al.*, 1980).

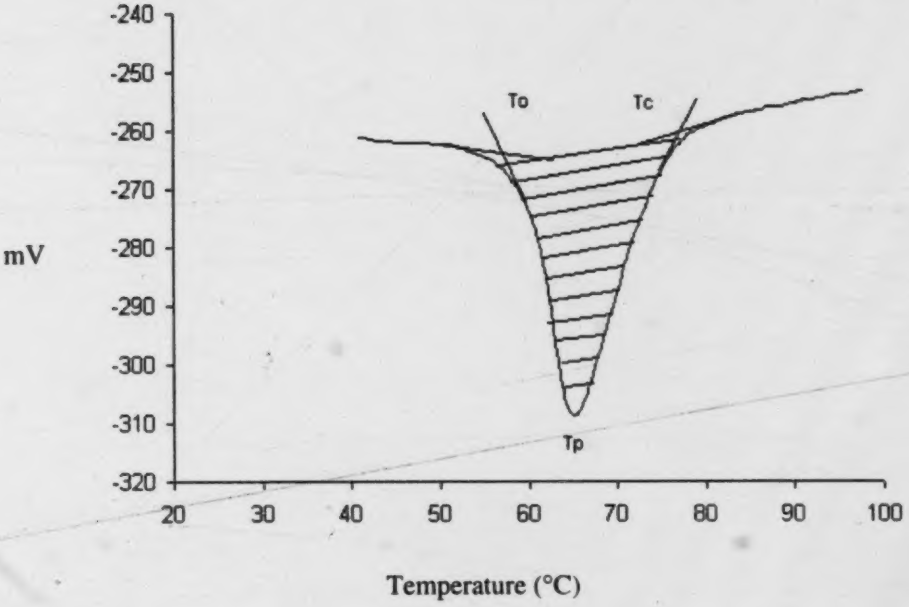
For all types of potatoes, after storage for 7 d, the gelatinization temperature of treated and untreated samples both stored in air and vacuum significantly increased by approximately 2°C ($p<0.05$), compared with that of freshly prepared samples. There was no significant difference in gelatinization temperature among stored samples packaged in air and vacuum. The onset of gelatinization (To) was found to be higher ($P<0.05$) by

Table 3.8. Thermal properties of starch isolated from raw potato strips before and after storage under various packaging conditions for 7 d at 4°C

Potato			Endotherm Temperature (°C)*			ΔH*
			To	Tp	Tc	kJ/kg dry starch
Group 1 (76% moisture)	Tuber 1	Fresh	54.33	63.05	73.48	10.65
		Untreated	58.05	64.58	74.22	11.41
		Air (treated)	57.99	62.22	74.92	11.46
		Vacuum (treated)	58.00	64.7	74.34	10.01
	Tuber 2	Fresh	53.20	59.69	70.70	10.65
		Untreated	56.95	62.58	71.25	11.19
		Air (treated)	56.41	62.22	72.19	11.75
		Vacuum (treated)	56.99	62.89	72.01	10.11
Group 2 (81% moisture)	Tuber 1	Fresh	56.17	61.82	73.28	12.74
		Untreated	56.56	62.87	72.95	12.86
		Air (treated)	55.86	62.81	72.03	11.70
		Vacuum (treated)	55.90	63.29	74.06	11.17
	Tuber 2	Fresh	55.94	61.72	72.27	12.36
		Untreated	56.48	63.12	74.92	12.26
		Air (treated)	55.78	63.36	73.67	11.8
		Vacuum (treated)	55.72	63.75	75.35	11.44
Group 3 (83% moisture)	Tuber 1	Fresh	55.39	61.87	73.90	11.62
		Untreated	56.72	63.83	76.64	11.91
		Air (treated)	55.55	62.97	74.61	11.39
		Vacuum (treated)	56.45	64.53	77.29	11.14
	Tuber 2	Fresh	56.95	63.14	73.91	11.32
		Untreated	58.20	65.07	76.17	11.62
		Air (treated)	56.56	64.69	76.41	11.31
		Vacuum (treated)	57.42	64.8	75.47	10.64

* Means of three measurements

Figure 3.1. A DSC curve showing gelatinization characteristics of an isolated starch. Tangents show the method used to calculate onset temperature (To), peak temperature (Tp) and conclusion temperature (Tc). Shaded area represents the transition enthalpy, ΔH.



about 3-4°C for stored Group 1 potato strips, compared with fresh samples, but no significant difference for To for stored Groups 2 and 3 potato strips. There was no significant difference of gelatinization temperature ranges (Tc-To) between fresh and stored strips for all types of potatoes.

Starch is a semi-crystalline entity, which contains both crystalline and amorphous regions. In amorphous regions, molecules do not have any particular order, swell readily and are more active. Molecules in crystalline regions have limited swelling and are more resistant to chemical attack (Leach, 1969). On heating, the optical properties of starch (e.g. birefringence) and its x-ray diffraction patterns are retained until temperature reaches gelatinization point, at which point the crystalline organization is lost and the granules swell irreversibly to form a viscous paste. The observed DSC transitions relate to starch gelatinization in that it can reflect a change or rupture of inter- and intra-molecular bonding (Wright, 1984). It is the large gain in enthalpy, in going from the ordered to the random conformation, that offsets the attractive stabilization forces (hydrogen bonding) occurring in the crystallites (Biliaderis *et al.*, 1980).

Tp, Tc and ΔH represent the fusion of starch granule structure. Peak temperatures also coincide with melting of the most perfect crystallites of the granules which is facilitated by amorphous swelling (Biliaderis *et al.*, 1980). Higher peak temperatures have been interpreted as indicative of greater resistance to gelatinization and indicate a more ordered crystalline structure (Biliaderis *et al.*, 1980; Hoover and Sosulski, 1985; Leszkowiat *et al.*, 1990). A high degree of crystallinity imparts structural stability, making the granule more resistant to gelatinization.

Therefore, increases in gelatinization temperature found in stored potato strips suggested the reorganization of starch molecular structure inside the granules. An increase in transition temperature may reflect a decrease in the destabilization (or stable amorphous region) effect of the amorphous region on the melting of starch crystallites during gelatinization (Leszkowiat *et al.*, 1990). It also suggested that a smaller proportion of amorphous regions could cause an increase in transition temperature because it could result in less effective swelling and crystallite destabilization (Leszkowiat *et al.*, 1990). A high gelatinization enthalpy also suggests a more stable granule structure due to greater crystallinity (Kim *et al.*, 1995).

It was found that an increase in gelatinization temperature is associated with several factors. Nonionic soluble constituents such as sugars and other polyhydroxyl compounds elevate the gelatinization temperature, as compared with water (Wright, 1984).

Transition temperature also increases after starch was applied heat-moisture treatment due to the transformation of starch crystalline structure from B type to C type (Kulp and Lorenz, 1981).

Starch from potato tuber has high phosphate content ranging from 0.05-0.08%, most of which exists as phosphate ester substitutions on anhydro glucose molecules, close to amylopectin branch points or in the bulk amorphous regions (Vasanthan *et al.*, 1999). Changes in starch properties were observed when there was cross linking between three molecules of phosphate and Ca^{2+}, and a higher gelatinization temperature was expected (Yamada *et al.*, 1986).

The crosslinked starches have slightly higher Tp, although To and Tc were unchanged. Lower ΔH was observed probably due to the increase in the rigidity of the crosslinked starches caused by intermolecular bridging, together with the restricted swelling (Wootton and Bamunuarachchi, 1979). However, it is unlikely that crosslinking of Ca^{2+} or Mg^{2+} relocated from cell wall materials to starch cause the increase in transition temperature because, from previous experiment, Ca^{2+} and Mg^{2+} contents in cell walls and starch of stored samples did not change during storage at low temperature. Therefore, other factors, more likely the changes in starch molecular structure during storage, could cause the higher gelatinization temperature.

A more plausible explanation for the results in the present study was a change in starch molecular structure during cold storage of potato strips, caused by as yet unknown factors, resulting in the increase in gelatinization transition temperature. According to Schoch (1969), starch retrogradation can occur within the ungelatinized starch granule, especially in high swelling starches like potato. Further heating allows association of molecules within the granule network, resulting in a more crystalline structure as evidenced by changes in the x-ray diffraction patterns. Gelatinization temperature was inversely related to swelling. This could explain the increase of gelatinization temperature in stored potatoes.

Changes in enthalpy were different among stored samples. Increased enthalpy by about 1 J/g in untreated and treated samples was found ($p<0.05$) on starch isolated from Group 1 potatoes. For Group 2 potatoes, there was no change in enthalpy of the untreated sample after storage, but a decrease ($p<0.05$) in enthalpy was found in treated-air packaged samples and vacuum packaged samples. Similarly to Group 1 potatoes, an increase in enthalpy, about 1 J/g, was found on untreated and treated samples stored in air packages. In general, enthalpy of vacuum packaged samples remained unchanged (for Group 3 potatoes) or slightly decreased (for Group 2 and Group 1 potatoes), although an increase in gelatnization temperature was found.

The ΔH value can account for a combination of energy required for several processes during gelatinization. At high water levels, the ΔH value can involve granule swelling, crystalline melting and extensive hydration of starch molecules (Biliaderis *et al.*, 1980; 1990). Cooke and Gidley (1992) indicated that the gelatinization endotherm is largely due to the melting of double helices. Therefore, the enthalpy would reflect on the degree of double helical packing. The higher the enthalpy, the higher the proportion of double helices or the crystallite packing. However, in some cases of high crystallinity starch, when double helices exist in the amorphous region of the starch, they may become easier to gelatinize and swell, therefore, reduced enthalpy is found (Vasanthan *et al.*, 1999).

Starch granule swelling is known to begin in the relatively mobile amorphous region composed of free amylose/amylopectin chains and amylopectin double helices that are not involve in crystal formation, and in the rigid amorphous regions close to branch points but immediately adjacent to crystalline regions (Vasanthan *et al.*, 1999). This process requires some energy and it will affect the net ΔH value from DSC test.

More detailed thermodynamic measurements are required to provide an adequate understanding of the processes contributing to the enthalpy factor of the gelatinization process (Biliaderis *et al.*, 1980; 1990). On the other hand, the ΔH value can represent the properties of the original crystallites. In the presence of limited amounts of water, decrease in the gelatinization enthalpies have been pronounced. A lower degree of disorder achieved by the starch during gelatinization of concentrated starch/water systems was proposed as the reason for the decrease in ΔH values (Stevens and Elton, 1971). From the results of the present experiment, there was no significant change in the ΔH values of the stored samples, although the transition temperature (Tp) noticeably increased from that of the starch of the freshly prepared potato samples. If a change of starch crystallinity occurred during cool storage, the gelatinization enthalpies would not be used as indices of starch crystallitnity because they represent the net thermodynamic qualtities of different events: granule swelling and crystallite melting (endothermic), and hydration and recrystallization (exothermic). This may help explain the inconsistent changes of ΔH in stored samples.

However, slightly lower or unchanged ΔH value was observed in samples stored in vacuum packages. If this represents the low swelling of granule, it is possible that the starch granule in vacuum packaging samples will swell less than in other samples. Separation of cooked potato cells, in this case, would be less than when starch granules swell extensively. Therefore, high lump formation in vacuum packaged samples could also be caused by changes in starch structure during storage.

Svensson (1971) found that in the hard tissues of stored pre-peeled potatoes after cooking, the middle lamella and the cell walls were not loosened and the starch was not swollen to the same extent as in normal tissues. The texture of the cooked potato is controlled by a number of factors, including starch and cell wall components, especially pectic substances (Reeve, 1972; Loh *et al.*, 1982; Javis *et al.*, 1992; Thybo *et al.*, 1998). During heat treatment, two dramatic changes occur: 1). starch gelatinization and swelling and 2). loosening of middle lamella and cell wall swelling (Potter *et al.*, 1959; Keijbets and Pilnik, 1974; Keijbets *et al.*, 1976; Hadziyez and Steele, 1979; Shomer 1995; Shomer *et al.*, 1995). The "swelling pressure" created during starch gelatinization is a major mechanism causing cell rupture and cell separation. The swelling pressure leads to a rounding-off of the cells thereby inducing cell separation (Reeve, 1972; Loh *et al.*, 1982; Javis *et al.*, 1992; Thybo *et al.*, 1998). If starch undergoes changing during storage as indicated by increased gelatinization temperature, a decrease in swelling of starch granule could reduce cell separation and increase formation of lump.

The results in the present study suggested that changes in starch properties occurred during storage of potato strips, which may be related to hard tissue formation. The amounts of lump formation were different between each type of potatoes, and somewhat different gelatinization temperature patterns of starch isolated from these samples were also found, thus pointing to the starch involvement in this phenomenon. Since the data from the DSC tests cannot explain changes in starch molecular structure in detail, more study must be done to elucidate changes in starch molecular structure.

3.4. Conclusion

The degree of lump formation after cold storage appeared to depend on the moisture content of the potatoes. Potatoes higher in dry matter content gave a higher lump percentage after storage for 7 d. Lump formation was also higher when samples were stored under vacuum packaging as compared to air packaging. A firmer texture of raw potato strips was found after samples were stored for 7 d at 4°C. However, no relationship between firmness values and lump formation was detected. A decrease in the content of water-soluble pectic materials was found to accompany hard tissue formation in pre-peeled potatoes after storage. The decrease in water-soluble pectic materials and the increase in lump formation were significantly higher when potato samples, especially those with low moisture content, were packaged in vacuum than those in air. An increase in gelatinization temperature of starch isolated from stored pre-peeled potato strips was found in both air packaged and vacuum packaged and treated and untreated samples, which may also contribute to the hard tissue formation.

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CHAPTER 4. CHEMICAL AND PHYSICAL CHARACTERISTICS OF HARD TISSUE

4.1. Introduction

In the previous experiments (Section 3.3.3 and 3.3.4), changes in pectin, starch and mineral content of pre-peeled potato strips were investigated. Some differences in water-soluble pectin content between stored samples and freshly prepared samples were observed. Some changes in the gelatinization temperature of starch fractions were also detected. These lines of suggested evidence that pectic substances in potato cell wall and middle lamella may play a major role, and starch a secondary role, in the formation of hard tissue in pre-peeled, refrigerated potatoes. However, the occurrence of hard tissue appeared to vary among potatoes of different varieties, with types of antibrowning treatments, and with applied packaging atmospheres. The mechanism of hard tissue formation is still not clear and appears to be multivariate. Thus far, experiments had been performed on whole fresh and treated potato tissues to evaluate differences among various treatments. In the experiments described in this Chapter, hard tissues were isolated from cooked, mashed samples and their chemical and physical characteristics were evaluated in comparison with regular tissues. Chemical parameters evaluated were contents of soluble and insoluble pectins, degree of esterification (DE) in the pectins, and mineral contents in cell wall and starch fractions. For physical characteristics, scanning electron microscopy (SEM) was used to evaluate surface appearances of cooked potatoes variously treated and of isolated hard tissues. It was anticipated that this study would provide additional chemical and physical evidence that might corroborate or disprove the results of the foregoing studies, Together, they might reveal the actual cause(s) of hard tissue formation, thus leading to a practical solution to the problem.

4.2. Materials and methods

Russet potatoes (24-25% dry matter) were Alberta grown, purchased from a local store and held at 4°C until needed.

Strips were prepared according to the procedure described in Section 2.2.1. CA/AA/EDTA was used as antibrowning agents following the procedure described in Section 2.2.1 and the samples were packaged in air and stored for 7 d at 4°C.

Lumps separated from the stored samples that had been steam-cooked and mashed, were used for all experiments in this section. In the separation procedure, deionized water was used to minimize the effect of interfering ions. Normal tissue (control) was prepared from mashing steam-cooked fresh potatoes.

4.2.1. Determination pectic substances

Alcohol insoluble solid (AIS) was prepared from hardened tissue or lump and normal tissue and was then extracted for water- and oxalate-soluble pectins. Residuals from water- and oxalate-soluble fractions were then extracted for water insoluble fraction with HCl, following the procedure in 3.2.3.1. Each fraction was determined for pectic substance content by the m-Hydroxydiphenyl reaction method as described in Section 3.2.3.1. The data were statistically analyzed.

4.2.2. Determination of mineral content in cell wall of hard and normal tissues

Cell walls were isolated according to the procedure described previously with slight modifications. After samples of freshly mashed potatoes and hard tissues were pulverized with deionized water, the slurries were cooled to room temperature and 1 g of pancreatin (Sigma Chemical Co., MO, USA) was added and mixed well on a magnetic stirrer. The samples were then incubated at 40°C for 4 h. Pancreatin (U.S.P. specifications, Sigma Chemical Co., MO, USA) would digest gelatinized starch in cooked potatoes into smaller molecules so that filtration to remove gelatinized starch was possible. After incubation, cell walls were separated from starch by sieving though a standard sieve No. 170 and washed several times with water. Isolated cell walls and starch were freeze-dried and stored in tightly screw-capped tubes until needed. Calcium and magnesium content determinations were performed following the method described in Section 3.2.4.2.

4.2.3. Determination of degree of esterification

Total pectin content (as anhydrogalacturonic acid) was measured using the method described by Walter *et al.* (1993). Duplicated 0.1-g samples of AIS were wet with 0.2 mL ethanol, placed in ice bath for 15 min, then 2 mL of cold, concentrated sulfuric acid were added and the mixture vortexed. An additional 2 mL of cold acid were added and the solution vortexed and then sonicated in an ice bath for 5 min. Samples were held at 4-5°C overnight. The viscous mixture were diluted to 50 mL with water and mixed. For analysis, 12.5 mL of diluted sample were pipetted and further diluted to 100 mL with water. The solution from the last dilution was used for the analysis of galacturonic acid content as described in Section 3.2.3.1d.

The pectin solution previously extracted from AIS was used for further determination of degree of esterification (DE) by enzymatic method described by Klavons and Bennett (1986). To hydrolyze methyl-esterified pectin, 25 mL of the pectin solution was added with 25 mL of 4N potassium hydroxide (analytical grade, Fisher Scientific, NJ, USA). Two replicates were done for each sample. The solutions were incubated at room temperature for at least 30 min. The pectin hydrolysates were neutralized with dilute phosphoric acid (analytical grade, Fisher Scientific, NJ, USA) to pH 7.5, using a pH meter. After adjusting pH, 1000 µL of neutralized solution was placed in 16 x 126 mm culture tubes. 1000 µL of 1 unit/mL alcohol oxidase (analytical grade, Sigma Chemical Co., MO, USA) solution was added to each tube. The tubes were gently mixed and incubated at 25°C for 15 min. 2000 µL of 0.02M 2,4-pentanedione (analytical grade, Sigma Chemical Co., MO, USA) in 2.0 M ammonium acetate (analytical grade, Sigma Chemical Co., MO, USA) and 0.05 M acetic acid (analytical grade, Fisher Scientific, NJ, USA) was added to each tube and mixed well by vortexing. The tubes were placed in a water bath at 58-60°C for 15 min, and cooled to room temperature. The absorbances were measured by Spectrophotometer (Spectronic 21, Bausch & Lomb) at 412 nm against a blank containing phosphate buffer of the appropriate concentration, pH 7.5, and dilute alcohol oxidase.

To calculate the % methyl ester content, the molar concentration of galacturonic acid/g AIS was divided into the molar concentration of methanol/g AIS and the result multiplied by 100.

4.2.4. Scanning electron microscopic studies of pre-peeled potato strips

Table potatoes (Canada and US grown), designated Group 2, were prepared for SEM studies. All samples were prepared in the same way as described in Section 3.2.3. Cooked and raw tissues (2x2x5 mm) from fresh and stored potato strips were prepared by cutting from the inner area of strips. Tissue sections were fixed at refrigerated temperature in 15% glutaraldehyde (analytical grade, Fisher Scientific, NJ, USA) in 0.1M phosphate buffer pH 6.8 for 24 h, and washed in the buffer for 20 min. The washing was repeated twice. Tissue sections were then dehydrated at room temperature by stepwise exposure to the following ethanol-buffer mixtures for 20 min intervals: 30%, 50%, 60%, 70%, 80%, 85%, 90% and finally 100% ethanol. The tissue sections were then critical-point dried. The dried tissue sections were then mounted onto sample stubs with double-sided adhesive tape and coated with about 20 nm of gold. Samples were then examined by a scanning electron microscope (Model 54. Cambridge Scientific Instrument Ltd. Cambridge, England) at an accelerating potential of 15 kV.

4.3. Results and Discussion

4.3.1. Pectic substance, Ca^{2+} and Mg^{2+} contents and DE of normal and lump tissues

Table 4.1 shows pectic substance content, Ca^{2+} and Mg^{2+} contents and DE of lump tissues in comparison to normal tissues. Higher ($p<0.05$) soluble and lower insoluble pectic substances were found in freshly cooked mashed potatoes, in comparison with lump tissue collected from stored potato strips after cooking and mashing which

Table 4.1. Some characteristics of hard tissues in comparison with normal tissues

Sample	Pectic Substances		DE (%)	Calcium Content (µg/mg)		Magnesium Content (µg/mg)	
	% Soluble	% Insoluble		Cell wall	Starch	Cell wall	Starch
Lump Tissue	21.25+4.99^{a}	78.75+4.99^{a}	69.49+3.66^{ns}	1.1582+0.31^{ns}	0.5494+0.07^{ns}	0.9292+0.16^{ns}	0.1379+0.04^{a}
Normal tissue	48.36+5.07^{b}	51.64+5.07^{b}	75.99+6.84^{ns}	0.7875+0.13^{ns}	0.6053+0.08^{ns}	0.7622+0.01^{ns}	0.3115+0.02^{b}

DE = degree of esterification

a,b : Mean scores in the same column not sharing the same superscript are significant different ($p<0.05$)

ns : No significant difference between means in the same column were low in soluble pectic substances but high in insoluble content. These results agree with those of previous experiments in that soluble pectin fraction in raw strips decreased after storage for 7 d at low temperature.

DE in lump tissue is considerably lower than that in normal tissue, though the difference is not statistically significant. Nevertheless, this indicates that more carboxyl groups were bound in lump tissue than in normal tissue. It has been suggested that preheating (Bartolome and Hoff, 1972) and chilled storage (Buescher *et al.*, 1976) could enhance membrane permeability, which allows cations to activate pectin methylesterase, followed by binding between carboxyl groups and the divalent cations, leading to a firmer texture.

The fact that there was a significantly higher insoluble pectins in lump tissue than in normal tissue, and that Ca^{2+} and Mg^{2+} contents were higher in lump tissue than normal tissue, albeit the differences were not significant, it is indicative that pectins play a major role in tissue hardening in stored pre-peeled potatoes. However, the hard tissue formation appears to be much more complex than can be explained by the role of pectins alone since other factors such as starch also come into play. More work is obviously necessary to elucidate the exact roles of these factors, which may lead to an effective practical solution to this problem.

4.3.2 Scanning electron microscopic studies of pre-peeled potato strips

Scanning electron microscopy (SEM) provides three-dimensional view of the microstructure of materials, which is beneficial for the understanding of physical and structural properties of biological materials. It is a very useful tool which has been applied in various areas of food and food product studies, e.g. food processing, food microbiology and food rheology (Pomeranz, 1976). It has also been shown that direct examination of food surfaces can make important contributions to solving some problems encountered by the food industry. Haard and Medina (1976) showed benefits of SEM to explain causes of a physical disorder called hardcore in sweet potato roots after chilled storage when it was combined with chemical studies. SEM has also been used to examine textural properties in some texture studies such as in the firming of cooked sweet potatoes affected by alum treatment (Sasaka *et al.*, 1999), in the firmer texture of vacuum packaged potatoes (Thybo *et al.*, 1998) and in the studies of mealy texture in different varieties of potatoes (McComber *et al.*, 1994).

In the previous experiments (Section 3.3.3), low water-soluble pectin in raw pre-peeled potatoes after storage seemed to suggest that modifications of the middle lamella might occur, resulting in its increased stability during cooking. Therefore, SEM studies were performed on potato samples in order to directly examine the textural characteristics of the stored and freshly prepared tissues to further elucidate the causes of tissue hardening.

The surface of specimen from a cooked fresh potato strip cut to the plane of the inside area showed parenchyma cells filled with gelatinized starch, while empty parenchyma cells were commonly seen in raw specimen, with some starch granules visible from some of the cells (Fig. 4.1B and A, respectively). Raw tissues were more easily sliced across the cells because of their turgidity (A), while cooked tissues were more pliable, thus most cooked cells remained intact (B). On cooking, the cell wall was softened and middle lamella was partially solubilized, while starch granules gelatinized

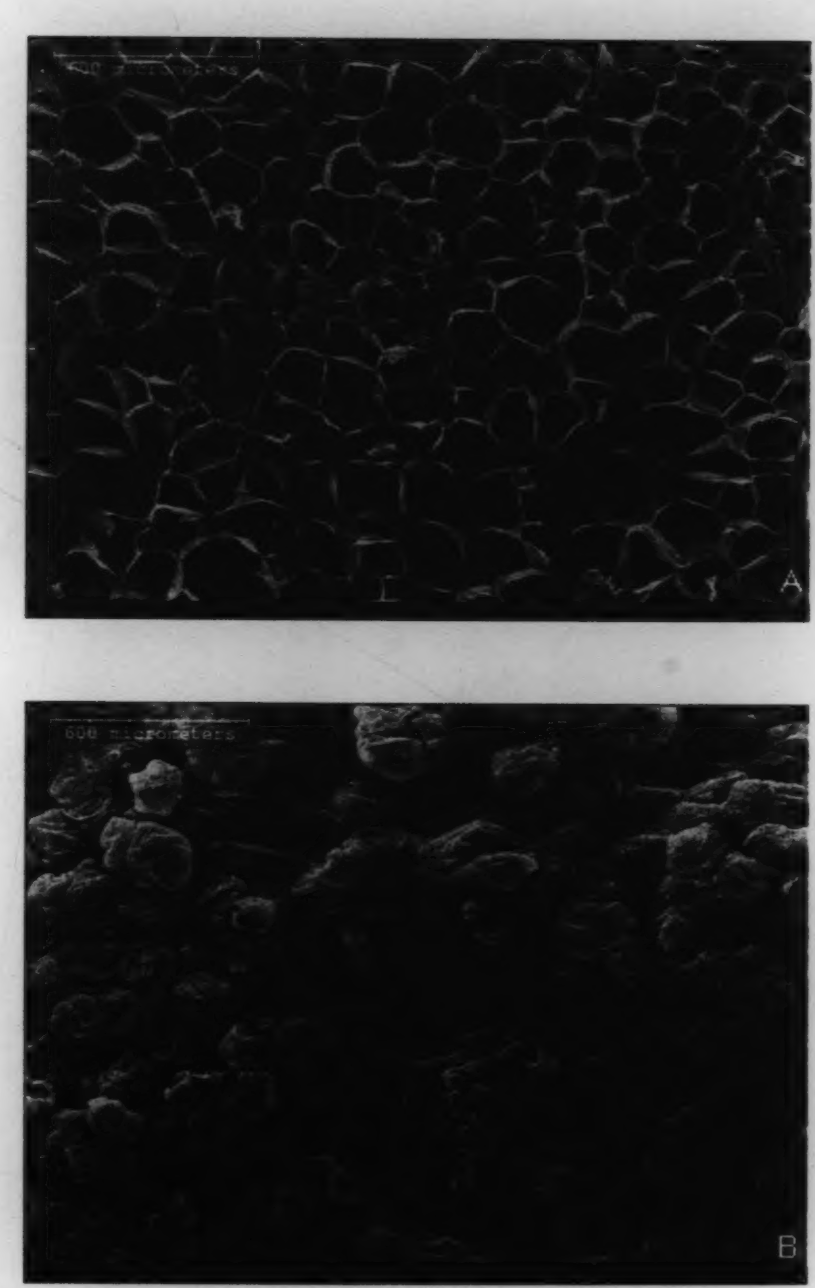
600 micrometers
A
600 micrometers
B

Fig. 4.1. SEM micrographs of Group 2 potatoes: (A) raw, untreated (control); (B) cooked, untreated (control) at x50 magnification

and expanded to fill and distend the cells, making them more easily separated from one another on mashing.

Fig. 4.2 shows structure of cell wall material and gelatinized starch. At high magnification, cell wall micrographs revealed aggregation of long fibrous structural material, probably cellulose microfibrils entertwined with one another, while gelatinized starch was shown as a sponge-like structure from a continuous matrix. The cooked cells were filled with gelatinized starch and appeared to be separating from one another.

Fig. 4.3 (A and B) illustrates the connection between two cells of freshly cooked potatoes. Although the two adjacent cells are seen to be linked together by strands of cellulosic materials, but the links are only partial and not appear to be strong. This is most likely due to the degradation of parts of cell wall and middle lamella during cooking. On the other hand, a much denser fiber matrix connecting two adjacent cells were observed from specimen prepared from antibrowning-treated samples packaged under vacuum (Fig. 4.4). These observations corroborate the previous results, in which high lump formation and low water-soluble pectins were observed in vacuum packaged samples when comparing with freshly cooked samples. Fig. 4.5 shows similar connective fiber matrix in cooked, untreated Groups 2 potatoes packaged in air and kept refrigerated for 7 d. The fiber matrix seems to be less dense, thus the cells could be separated more easily on mashing. A more completely degraded middle lamella can be seen in Fig. 4.6 (A and B) prepared from treated Group 2 potatoes, air-packaged and refrigerated for 7 d. No lumps were found in Group 2 and 3 samples, treated or untreated, even after 7 d refrigerated storage.

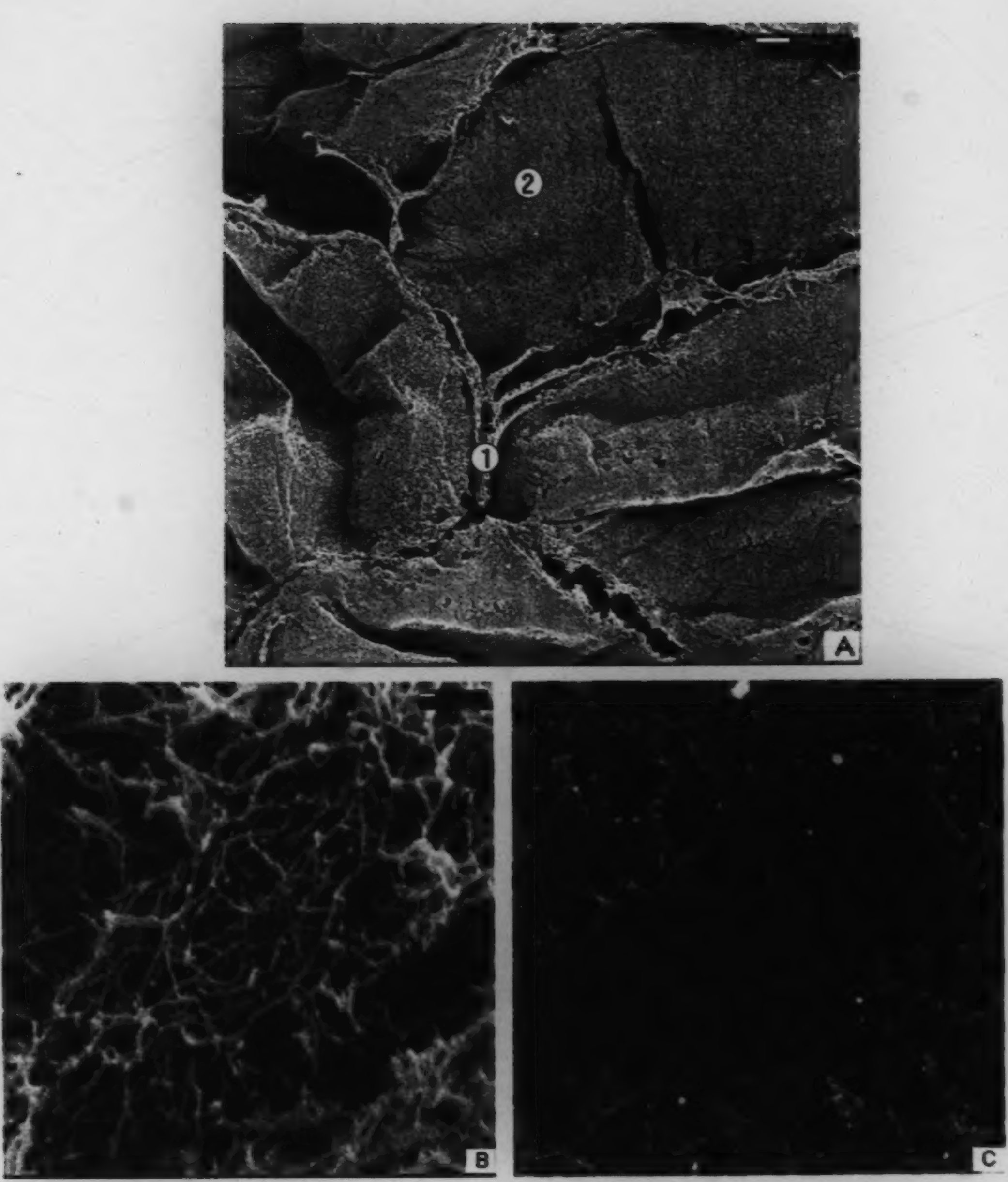

Fig. 4.2. SEM micrographs of Group 2 potatoes treated with AA/CA/EDTA and stored at 4°C for 7 d under air packaging at x500 magnification. (A) After cooking; (1) represents cell wall area, (2) represents gelatinized starch area. (B) shows cell wall area (1) at x30000 magnification (C) shows gelatinized starch area (2) at x30000 magnification

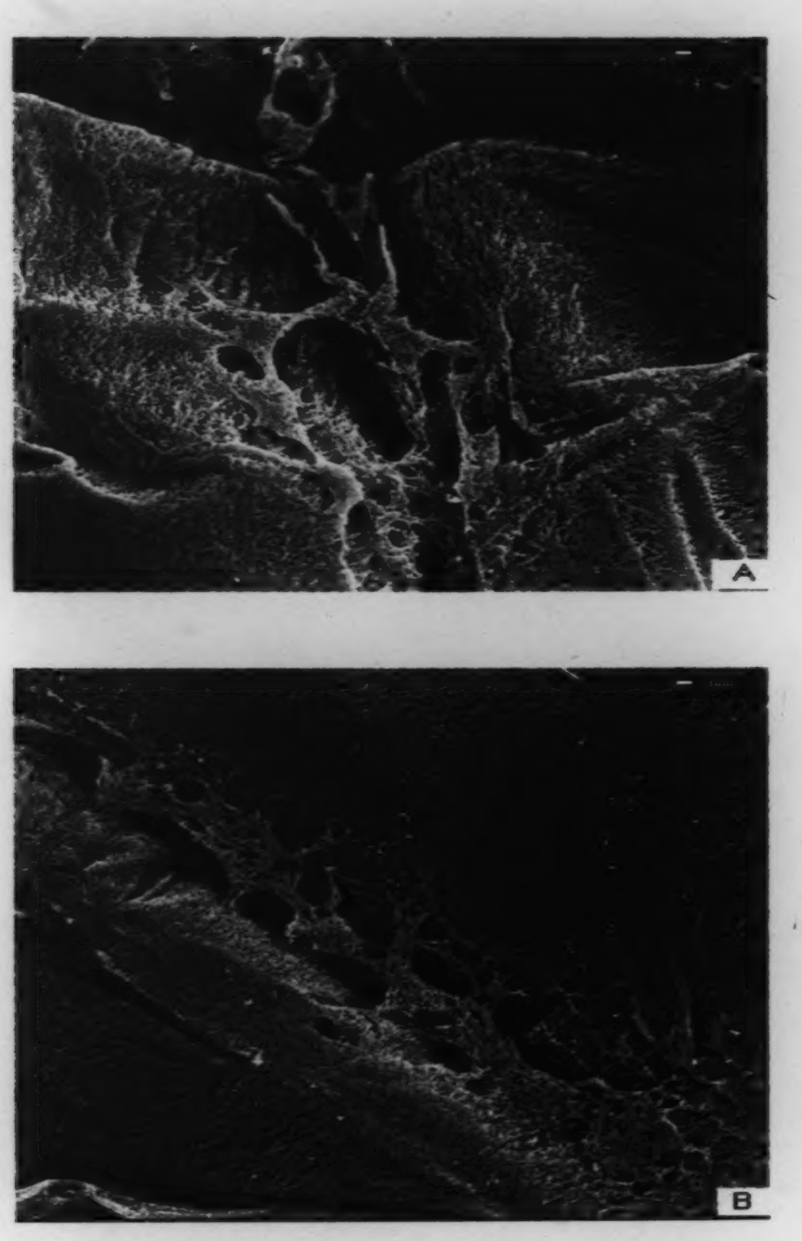

Fig. 4.3A and B. SEM micrographs of cooked fresh potatoes (Group 2), showing middle lamella area between two adjacent cells at x2000 magnification

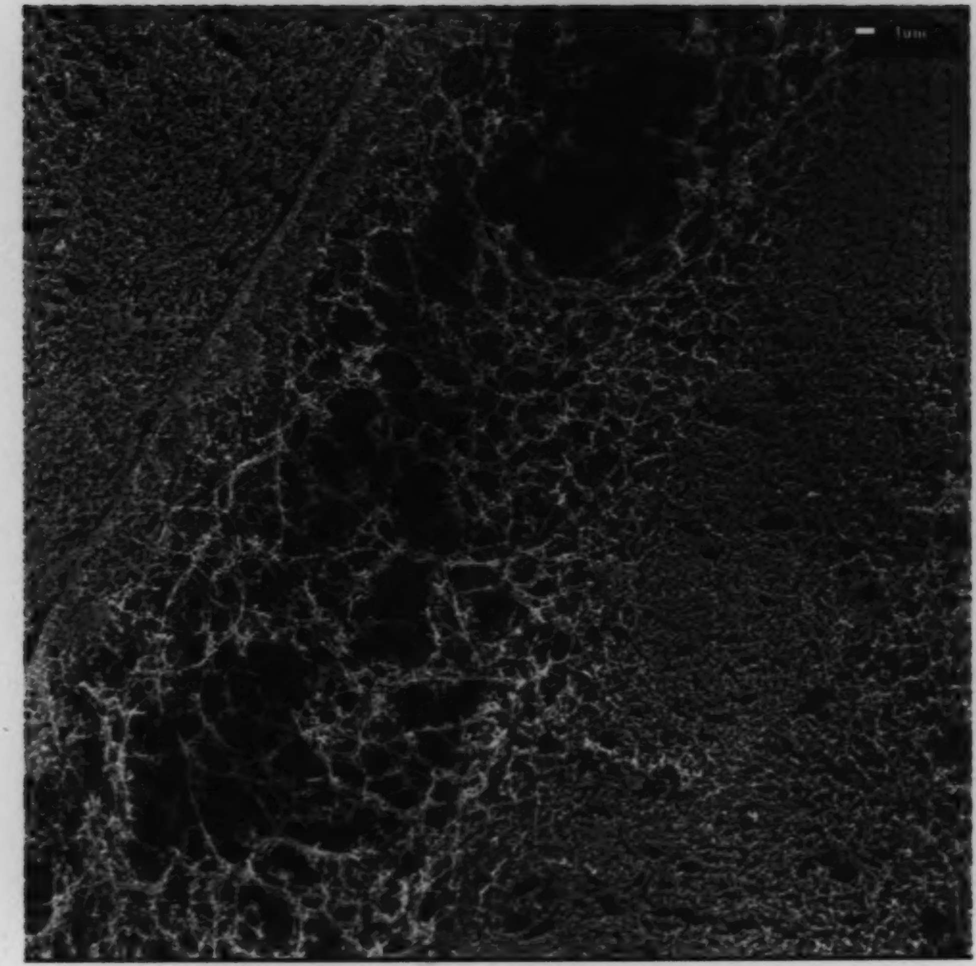

Fig. 4.4. SEM micrographs of cooked Group 2 potatoes treated with AA/CA/EDTA and stored at 4°C under vacuum packaging for 7 d, showing middle lamella area between two adjacent cells at x2000 magnification

Fig. 4.5. SEM micrographs of cooked untreated Group 2 potatoes stored at 4°C under air packaging for 7 d, showing middle lamella area between two adjacent cells at x2000 magnification

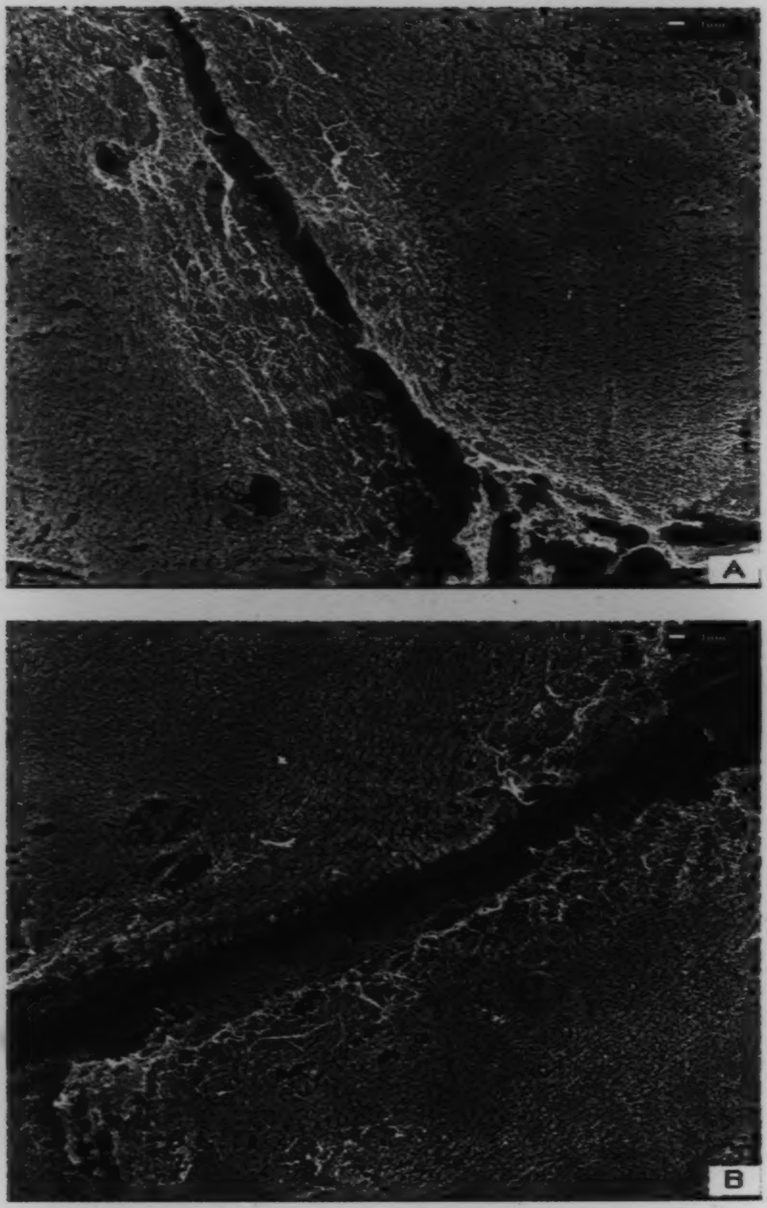

Fig. 4.6A and B. SEM micrographs of cooked Group 2 potatoes treated with AA/CA/EDTA and stored at 4°C under air packaging for 7 d, showing middle lamella area between two adjacent cells at x2000 magnification

Although, lumps were not found on untreated samples of Group 2 and 3 potatoes, it is possible that cold storage, especially when packaged under vacuum, could induce hard tissue formation since dense fiber matrices had been observed in these samples, though much less frequently when compared with those in Group 1 potatoes, in which high lump formation was found. Fig. 4.7A shows an overview of normal cooked tissue where cells can be easily separated, while Fig. 4.7B shows an area in which cells seem to be tightly bound together to form a lump.

Fig. 4.8 (A and B) shows a lump tissue separated from a treated, vacuum-packed Group 2 potatoes. The adjacent cells appear to be tightly bound together with just a narrow space indicating a boundary between them, even at high magnifications. Little degradation of cell wall and middle lamella in this tissue appears to have taken place during cooking, which corresponds to the high insoluble pectin content formed in lump tissues in the previous experiments.

Reduced solubility or degradation of cell wall materials could be caused by several factors. A disorder known as hardcore was observed when sweet potatoes were chilled and transferred to a non-chilling temperature (Buescher *et al.*, 1976; Buescher and Balmoori, 1982). This disorder is due to the formation of hard tissue of various dimensions after processing, which is similar to the problem found in potatoes except for the fact that hard tissue formation in potatoes occurs during cold storage. Buescher *et al.* (1976) found that less water-soluble pectic material, but higher sodium hexametaphosphate (SHMP) and hemicellulose were found in processed sweet potatoes exhibiting hardcore problems, while Daine *et al.* (1976) found an increase in protopectin in hardcore tissue and suggested that the hardcore was the result of a modification of

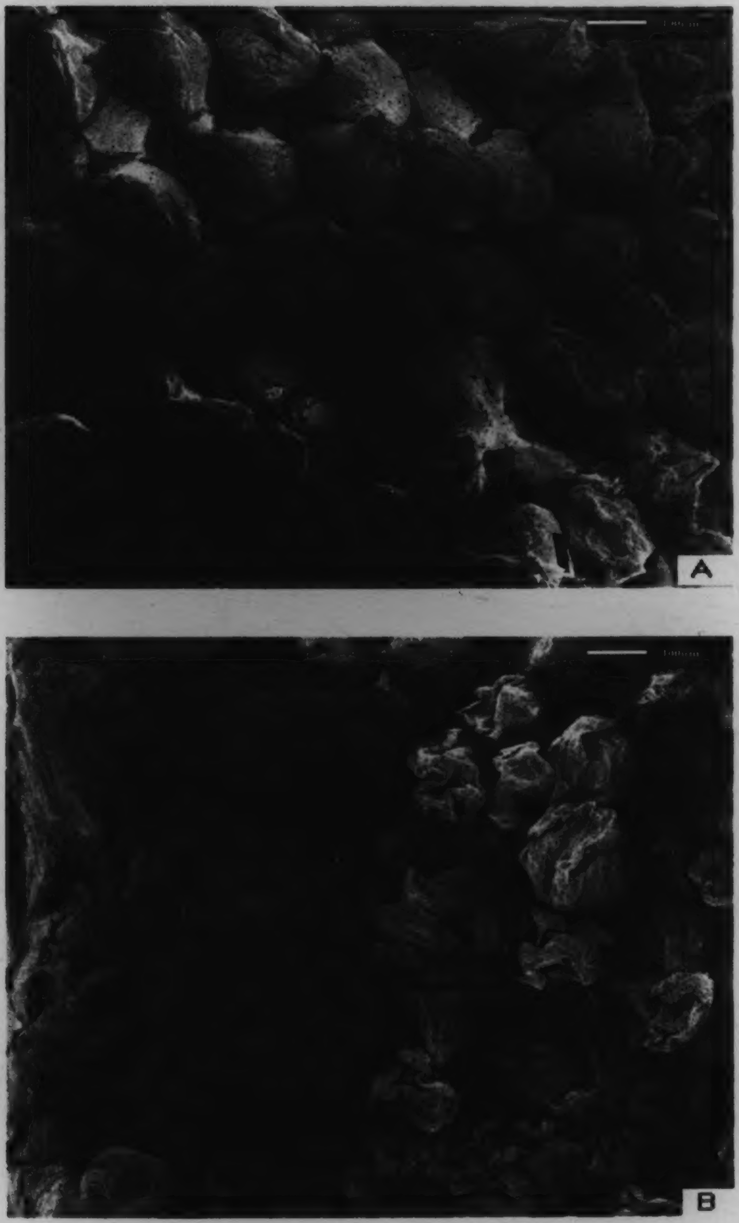

Fig. 4.7. SEM micrographs of cooked Group 2 potatoes treated with AA/CA/EDTA and stored at 4°C under vacuum packaging for 7 d at x75 magnification: (A) a typical surface view of cooked tissues; (B) an area indicating possible lump formation

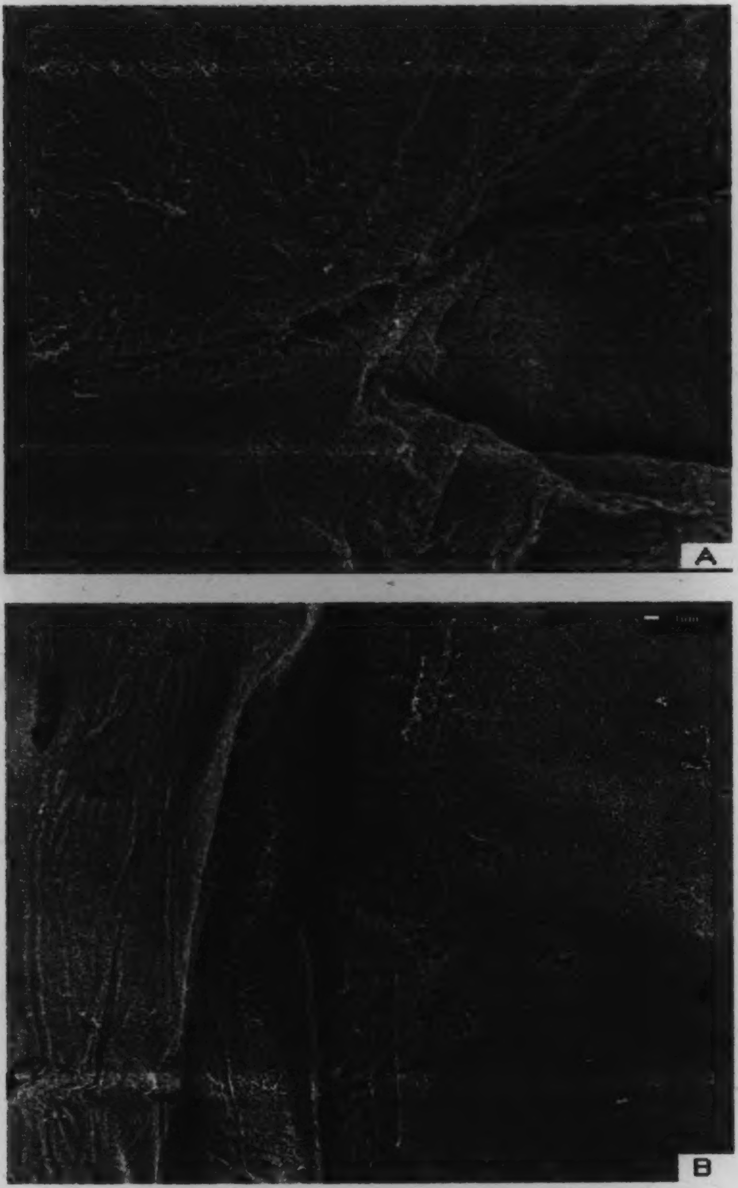

Fig. 4.8A and B. Lump tissue isolated from mashed Group 2 potatoes treated with AA/CA/EDTA and stored at 4°C under vacuum packaging for 7 d, showing middle lamella area between two adjacent cells at x2000 magnification

middle lamella. These results corroborate the findings in the present study. The involvement of pectic substances in preventing softening of chilled sweet potatoes was also supported by scanning electron microscopy which indicated that hardcore tissue had rigid cell-cell junctures even after cooking (Haard *et al.*, 1976).

4.4. Conclusion

A decrease in water-soluble pectic substances and an increase in water-insoluble fraction was found in lump tissues when compared with fresh potato tissues. Little difference in DE, Ca^{2+} and Mg^{2+} contents in the cell wall were found between lump and fresh tissues. SEM studies revealed the formation of dense fibrous materials connecting adjacent cells in samples of cooked potato strips stored for 7 d at 4°C under various packaging atmospheres The connective materials appeared to be more dense in lump tissue, preventing the cooked cells from being separated on mashing.

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CHAPTER 5. SUMMARY AND RECOMMENDATIONS FOR FUTURE STUDY

SUMMARY

Hard tissue formation is one of the major physical disorders of pre-peeled potatoes where affected tissues do not soften on cooking, making them unsuitable for consumption. The occurrence of hard tissue is a serious problem facing the pre-peeled potato industry. The causes of the hardening are still not clear. Therefore, the main objective of this study was to investigate the roles of some parameters responsible for the development of tissue hardening.

The results of the study suggested that a combination of mechanisms is responsible for the development of hard tissue in pre-peeled potatoes. Two major components in potatoes were involved in this problem: pectic substances and starch. A decrease in water-soluble pectic materials, accompanied by an increase in water–insoluble pectic materials were found to be a prominent factor in hard tissue formation in pre-peeled potatoes during refrigerated storage. Lower water-soluble pectins were also observed in cooked lump tissues when compared with normal tissues. Enzymatic modifications of water-soluble pectins during cold storage might be the cause of these changes. The modifications of pectic substances in the cell wall was clearly evident in SEM studies of potato samples, where dense fibrous materials connecting adjacent cells in hard lumps appeared to prevent the cooked cells from being separated on mashing. This dense fibrous materials could be pectic substances that had been strengthened and made insoluble through cross-linking of pectin carboxyl groups by endogenous ions such as Ca^{2+} and Mg^{2+}. This appeared to be corroborated by the fact that higher Ca^{2+} and Mg^{2+}

contents in cell walls and a lower degree of esterification (DE) of pectic fraction were observed in cooked lump tissues when compared with those of tissues exhibiting normal texture.

An increase in gelatinization temperature was also observed in starch isolated from pre-peeled potatoes stored at refrigerated temperature, which indicated structural changes in starch molecules. This might result in less swelling of potato starch granules, reducing the degree of cell separation, which might enhance hard tissue formation.

The present study also suggested that hard tissues developed during low temperature storage whether antibrowning treatment was applied or not. Other factors such as types of potatoes and packaging conditions also influenced hard tissue formation. Potatoes with higher solid content (or low moisture content) developed a higher amount of hard tissue than those with lower solid content. Packaging pre-peeled potato strips in vacuum or modified atmosphere with low O_2 concentration and high CO_2 concentration and storage at refrigerated temperature also appeared to enhance hard tissue formation.

RECOMMODATIONS

This study has indicated that tissue hardening is a complex phenomenon in which several parameters could be involved. Some important parameters have been identified but the exact mechanism of hard tissue formation is still not clear. More studies must be done to provide a clearer understanding of the problem. The following are some suggested further studies:

1. It was shown that amounts of hard tissue formed varied among potatoes of different varieties. This is probably caused by differences in their chemical composition.

Therefore, studies on potato varieties and hard tissue formation could provide useful information leading to an identification of suitable varieties for pre-peeled potato products.

2. Storage time prior to the processing of potato tubers could result in some changes in chemical components in the tubers, such as concentration of various fractions of pectic substances and the amount of starch. This could affect hard tissue formation during storage of the pre-peeled products. Therefore, a study on the effect of storage time of potato tubers before the pre-peeling process should be conducted.

3. Although antibrowning agents are not a major factor causing tissue hardening since untreated pre-peeled potatoes also developed hard tissues after cool storage, the present experiments indicated that different amounts of hard tissues were found when different antibrowning agents were used. A more thorough study on various types of antibrowning agents and their effects on pre-peeled refrigerated potatoes may identify suitable types of antibrowning agents for these products and identity the types of agents to avoid.

4. Changes in other cell wall components, besides pectic substances and mineral contents during refrigerated storage of pre-peeled potatoes, e.g. hemicellulose and phenolic substances, are also worth an examination. These components could also play a role in this problem.

5. Cell size, amylose and amylopectin contents and presence of organic acids such as phytic acid as related to the degree of hard tissue formation should also be studied.

6. Evidences indicated that some enzymes regulating the texture of potatoes such as pectinases and cellulases might be affected when potatoes are subjected to the pre-peeling process followed by prolonged storage at refrigerated temperature. Therefore,

investigation of the activities of these enzymes during storage would give valuable information.

7. Direct measurement of Ca^{2+} or Mg^{2+} binding capacity in potato cell walls as affected by cold storage would provide additional evidence supporting whether hard tissue formation is caused by a cross-linking of pectin carboxyl groups with divalent ions such as Ca^{2+} and Mg^{2+}.

8. It would be of interest to investigate starch granule swelling, cell separation and starch molecular structure as affected by storage time and conditions of pre-peeled potatoes. This would provide useful additional information regarding the effects of starch on tissue hardening problem.

The information obtained from these additional studies will provide a better understanding of the nature of the hard tissue formation, which may lead to the development of a practical solution to this important problem faced by the industry.